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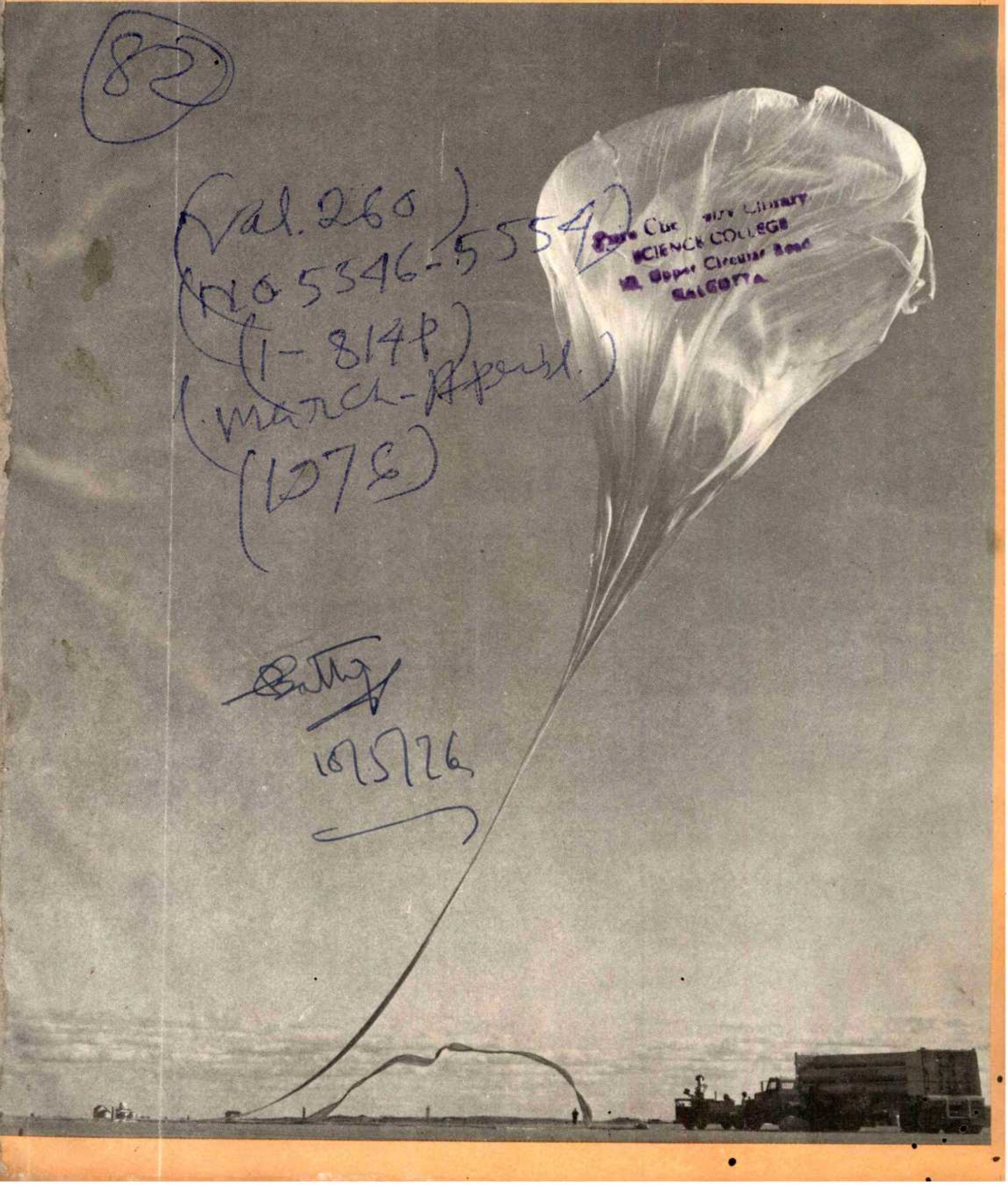

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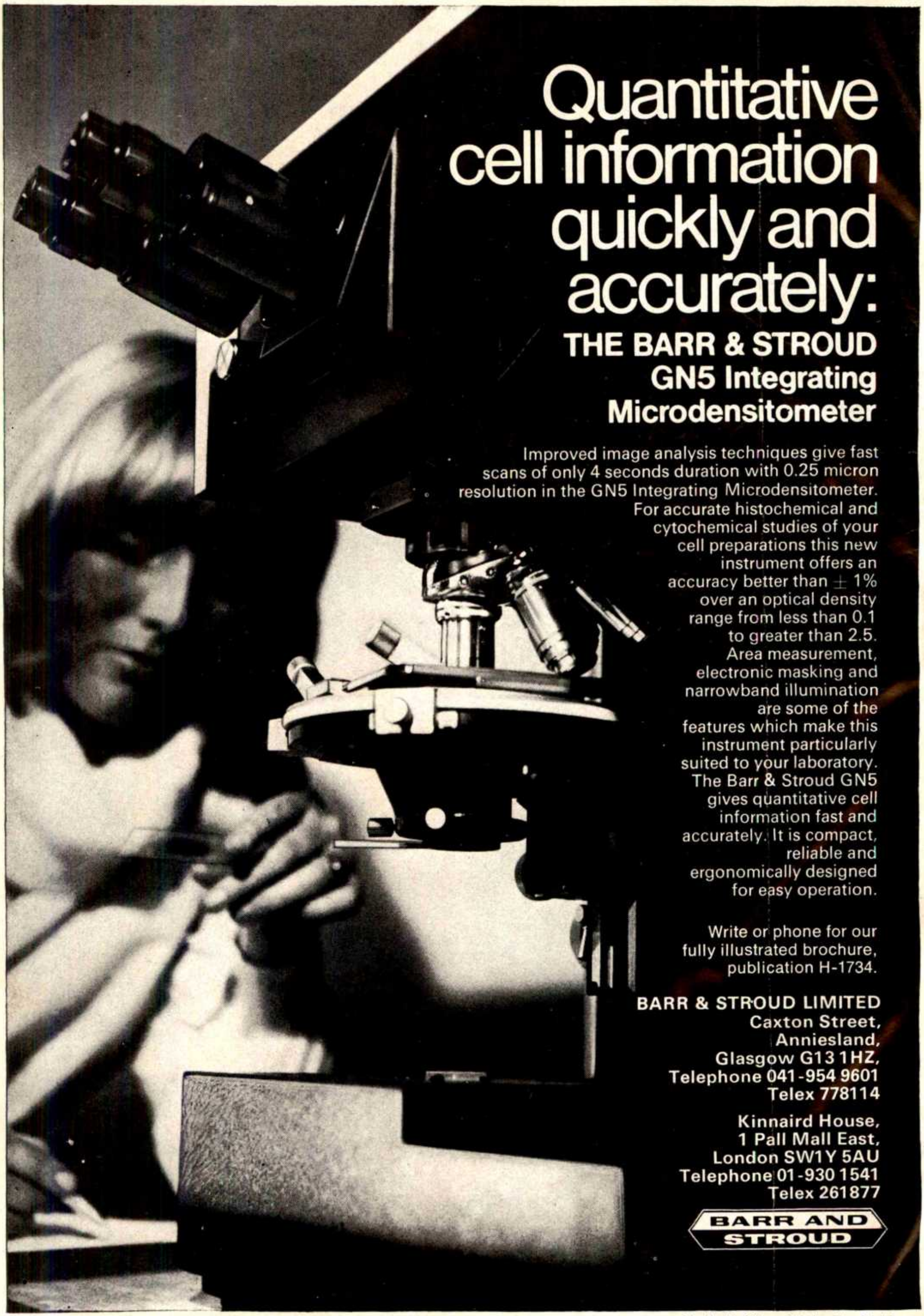

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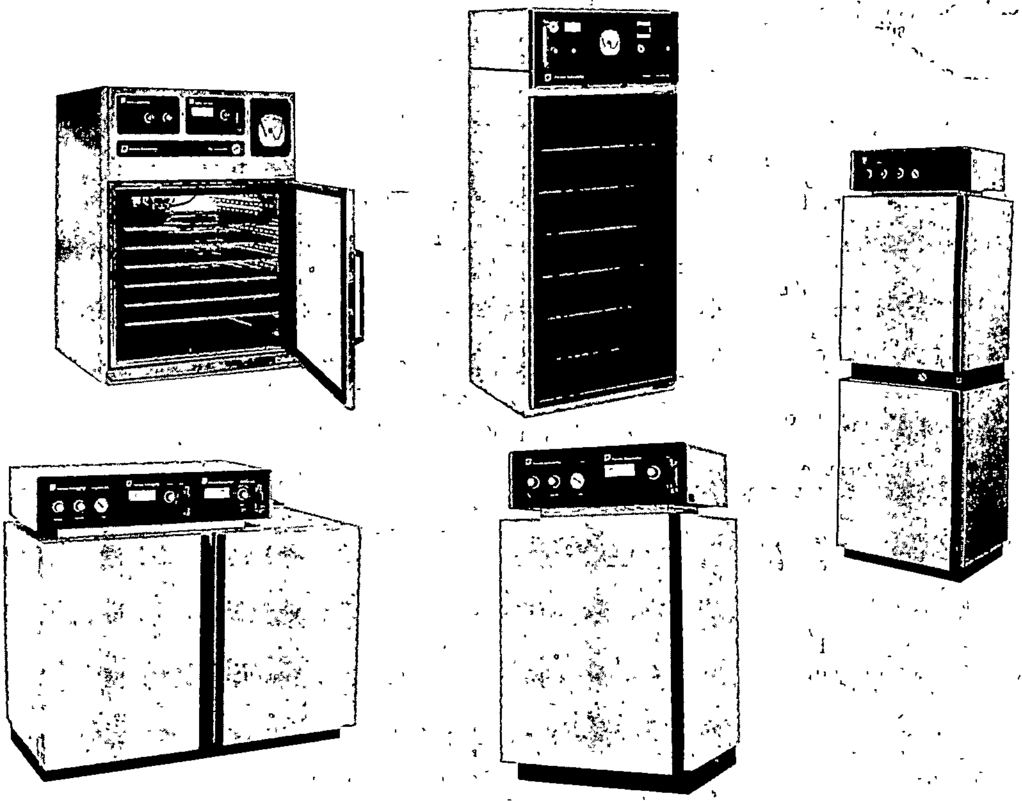

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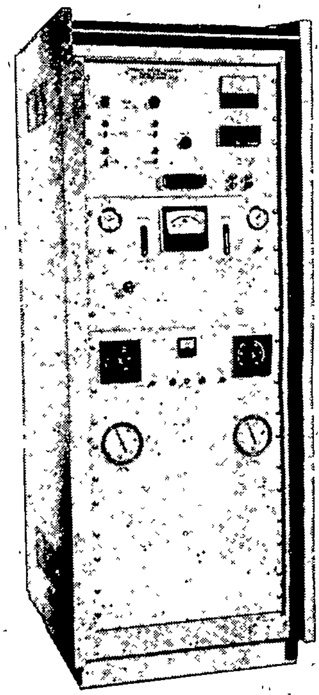

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Cover picture
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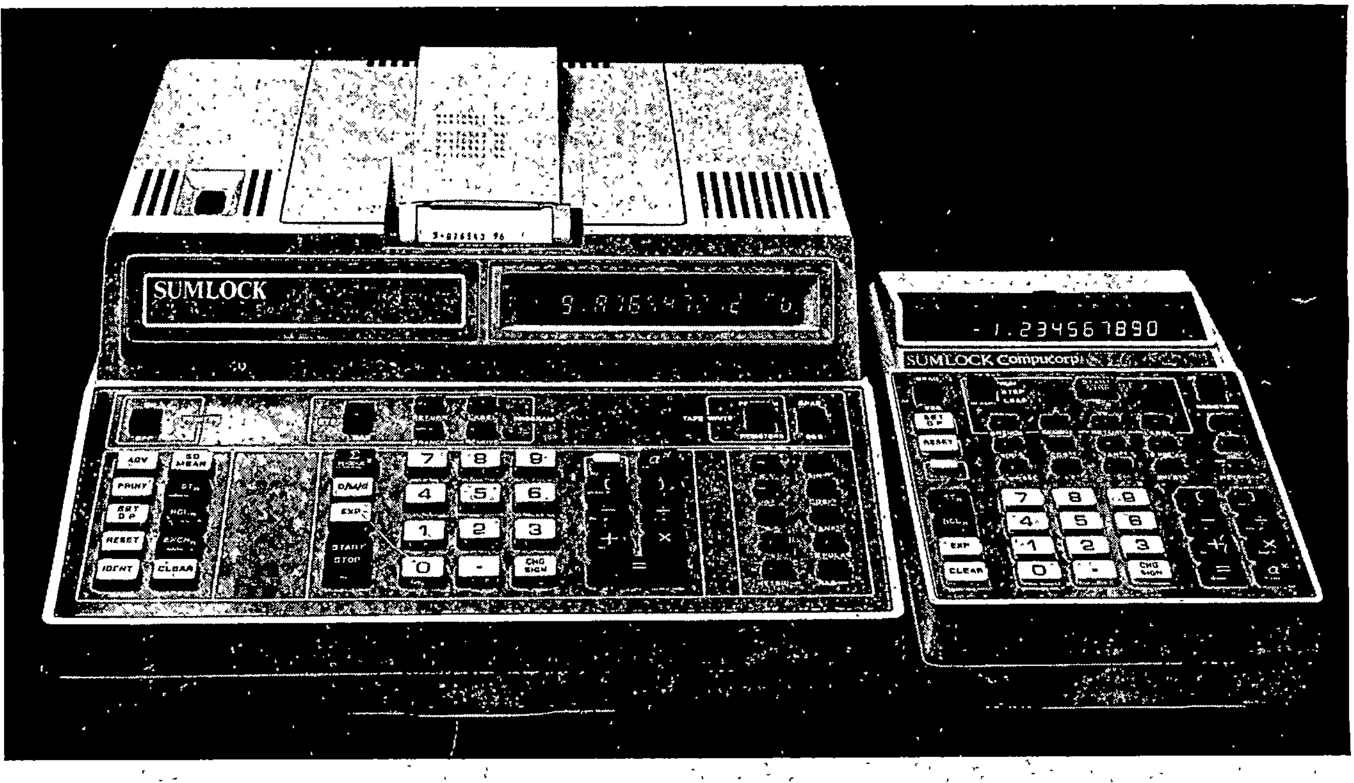

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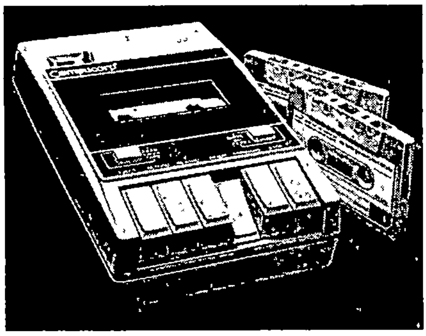

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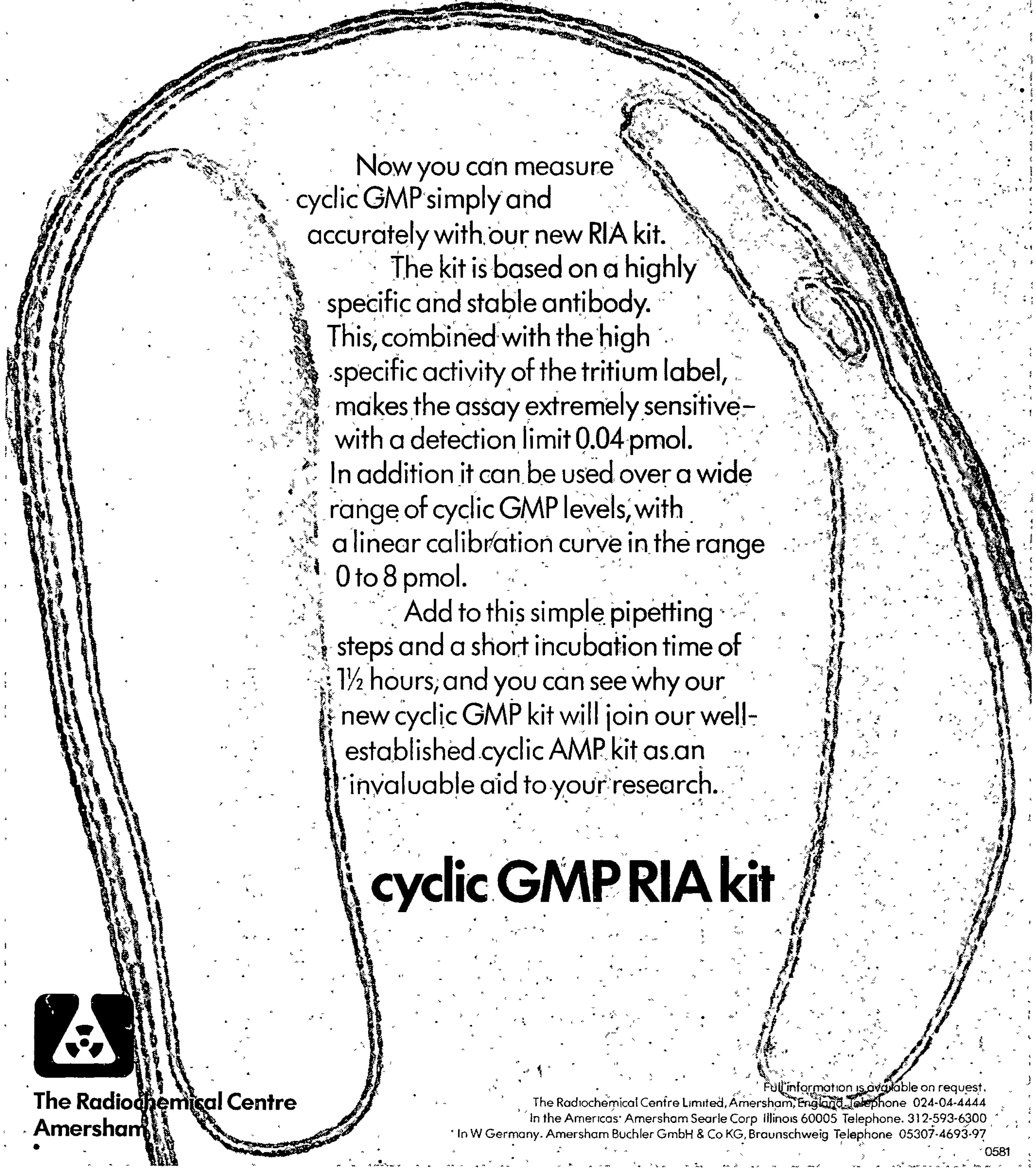

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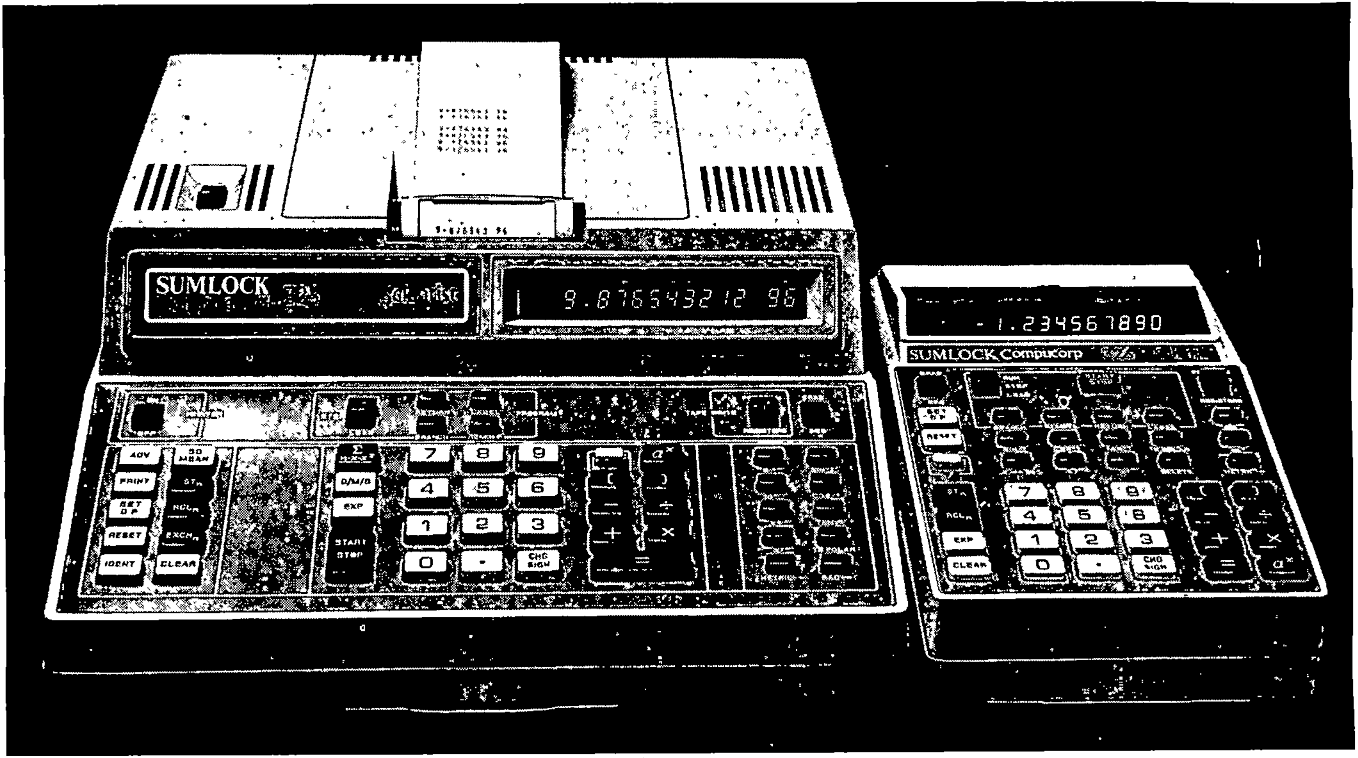

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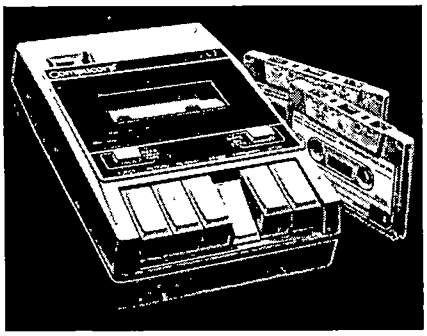

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The Mayan Temple of the Giant Jaguar, taken from within a second Mayan temple; Tikal, North Guatemala.

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Guide to authors

- Articles are up to 3,000 words in length with at most six displayed items (figures and tables) and may either be reports of major research developments in a subject or broader reviews of progress.
- Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four displayed items (figures and tables).
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nature

March 4, 1976

Getting down to business?

From the laboratory to the production line; a particularly difficult route, it seems, for British industry. First we look at the question of academics becoming industrialists.

OVER the past eight years the Wolfson Foundation has been supporting university-based research and development projects aimed at helping the national economy. Four million pounds has been made available for the period 1968–78, and sixty projects have been supported. A new round of grant applications is being solicited at present and a further £2 million will be made available to university groups with proposals which "have a real bearing on industrial production". Some of the more successful of those who have earlier held grants from the Wolfson Foundation were in London recently to talk about their experiences.

Success can be difficult to measure. Some units have actually managed to generate sufficient income from royalties, consultancies and contract-work to guarantee them a life of their own when the Wolfson support runs out. Others have found that the work they were doing was (and this phrase came up more than once) too far ahead of its time in the industrial context; though this may not be unsuccessful in a broader context. But one field in which the admirable efforts of the Wolfson Foundation have as yet to bear much fruit is in persuading members of the academic community to stand full-square behind their own inventiveness and launch a company to take advantage of the business opportunities with which they are presented.

The foundation would no doubt argue that it has never been its role to encourage university staff to forsake the campus, nor was the money it supplied to be seen as venture capital. "We are not a merchant bank", said Lord Zuckerman, one of the foundation's trustees. And yet it needed only a little goading before he would add that academics had too little courage and were far too sessile when it came to considering industrial prospects. What he had hoped for was an increase in the number of small companies working from a technological basis of high quality.

Spin-off industry is, of course, a thoroughly American concept, and it would be foolish to ape the practice simply because it comes from a country with a long record of academic–industrial rapport; after all, Route 128 has had its hard times. There are, however, some very positive reasons why universities should spawn more independent business.

Perhaps the most mundane reason is that if university and private business compete for the same work, it is possible for universities to fudge the overheads issue. This is not necessarily done malevolently—many at the Wolfson meeting were seriously questioning how they could make a realistic estimate of overheads—but the mingling of functions in a university environment is not, one suspects, a very healthy way of maintaining good university–industry relations. Nor, for that matter, is the use of students to provide the labour, in the long term.

A more important reason to encourage more spinning-off is the pressing need to demonstrate to students the role of private industry and the profit motive in a mixed economy. If he sees his teachers and advisers going through the motions of recommending to him the virtues of the private sector whilst themselves clinging to the security of the common-room, even though sitting on a fine business prospect, the advice can hardly bear much conviction.

There is much scope for misunderstanding in industrial–university collaboration. Each can so easily believe the other to be unreliable in delivering the goods. In particular the academic often see "The Board" as a remote and fickle body, prone to abandon high technology at the slightest sign of recession or in the event of a take-over. Surely, then, the answer is often in the academic's own hands: form his company, control his own board, then at least it will not be possible to blame anyone else if things go wrong—nor will anyone else reap the profits if things go right.

To the vice-chancellor the thought of losing good staff is obviously a worrying one, but there is no real reason why the long-term benefits should not be substantial. Much is talked and little is yet done about bringing scientific and engineering departments into closer liaison with industry for teaching purposes, both at the undergraduate and postgraduate level. Whom better to start such schemes with than managers of companies put together within, and spun off from, universities?

The development gap

. . . but what is NRDC doing? Professor S. D. Smith, who is both Professor of Physics at Heriot-Watt University and Chairman of Edinburgh Instruments Ltd, writes on the problem of support for development.

The Science Research Council (SRC) dispenses about £100 millions for research purposes each year. The community using it almost certainly contains the greatest concentration of academic brain power, but only a minor proportion of innovative, entrepreneurial and even practical ability, in the UK. But is there a mechanism to turn the practical results of this national investment efficiently into industrial products that could contribute to the economy?

Most people would, I submit, say no. One body representing academics, the Standing Committee of Professors of Physics, recently submitted a document to a Parliamentary Committee on Science and Technology stating that "most university investigators would prefer to invent nothing than something which might fall into the hands of the National Research Development Corporation" The organisation which automatically acquires rights for the exploitation of any activity currently funded by the SRC.

The appropriate development funding to take a device from a research laboratory to production line can be several times the cost of the original research Certainly obtaining original research results is easier than making the equipment viable on a routine basis; for example, the laboratory research yielding the vertical temperature sounder known as the Selective Chopper Radiometer for the satellite Nimbus 4 cost about £40,000 while the development programme to engineer it for space cost nearer to £200,000.

One major problem is that NRDC funds for development extend only to about £5 millions per annum—insufficient, on the above arguments, to develop even a small proportion of "device-successful" research originating from the SRC. Even more important, NRDC officials themselves differ on whether or not the NRDC is there to encourage innovation and help British industry. But if its specific objective is not to help British industry, what is its purpose at all? Not only does modest development not seem to meet with favour in the NRDC; under the harsh financial terms the NRDC attempts to extract from companies and investors, it seeks far ranging rights and then attempts to get its money back on *each* specific project by way of royalties at the rate of up to 12% per year on the unit selling price.

There are two immediate difficulties with this. Firstly, only about one out of ten projects may be *financially* successful, although a larger proportion may be technically successful. Secondly, the burden of a 12% royalty, being very large in proportion to the possible profit margin (probably about the same) in any competition with (in particular) American companies, can drive the selling price above a realistic level. I also suspect that the principle of trying to recover money *rapidly* on each specific project is wrong.

A related problem is that the NRDC only gives 50% of development funds. For both small and large industry today, this poses an almost insuperable problem of where to find the other half. A further condition invariably includes giving *all* rights and patents to NRDC: this is quite inconsistent with partial funding! The harshness of such terms make NRDC a worse prospect than a merchant bank—the more so because of the wearisome negotiations that drag on for months into years with innumerable wrangles with officials, accountants and so on. Thus in many ways it does *not* fulfil the role of financing truly developmental projects. There is only one saving grace; the NRDC shoulders the burden of loss in event of a failure. As I see it, the only way for a company to make a commercial success of an affair with the NRDC is to extract as much money from them as possible and then declare the project a failure.

So, how does a successful development occur at all? I believe that the British scientific instrument industry is almost in a position of not being able to progress without that element of luck or good fortune characterising all science and industrial development. The state of the laser industry and of the optical components industry and subtechnologies shows this clearly. The fact that things do happen is, I believe, quite often due to spin-off from some large project which carries with it a necessity for engineering and technical development. The development of some component or system then takes place, initially through what I would call "tame orders". Such an order a company can cope with; it can carry out the work for a given amount of money. This must be responsible for much more development than the NRDC route.

Such procedure brings into question the role of agencies beyond the SRC/NRDC area and in particular the Ministry of Defence and so on, where the method is commonly applied. There are two disadvantages: this source of support is not generally accessible, as of right, as a route for development to companies working outside the "defence" area or by the university research community; and, second, it is dictated very much by the whim of current "defence" thinking. I believe that there is case for optimising the rules of the games for research and development expenditure in the direction of helping the development of devices when they lead towards industrial products. Opponents would argue that many of these things will not be economical anyway; but we are spending the money in any event with equally little economic reason.

Why don't we, as a matter of urgency and seriousness, optimise development routes? Some moves would require political initiation, but the terms of reference are not so difficult to envisage. With large projects (like the SRC's Laser Laboratory), some integration into British industrial effort should be attempted as a matter of policy. It must be wrong for the good of the nation to proceed into new technical areas independently of encouraging improved technology in the country. Then, instead of the NRDC, perhaps we require a new branch of the SRC, that can commission development work or pre-production orders and coordinate this activity with the pure science expenditure. Awards could be made and judged against a serious review of technical performance with the sanction of no more funds against inefficiency. Where industry is involved a shared support of the order 80% from SRC to 20% from industry might be right, combined with a liberal and flexible attitude to rights and patents.

To cries of "featherbedding", the answer is not to have no bedding at all. An energy gap may be a source of devices in semiconductors; a development gap is no source at all. This nettle must be grasped at the level of politics.

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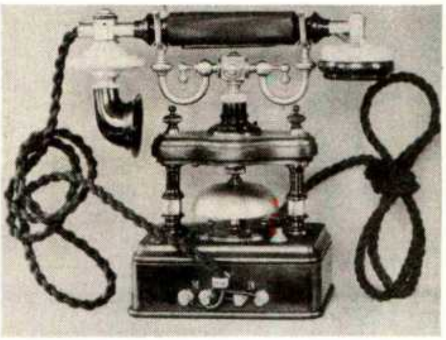
Speaking up for the telephone

Next week sees the centenary of the telephone. Below, a look at telecommunications in Britain



IT MAY be going too far to suggest that, in telecommunications, the pace of research and development may have all but outstripped our capacity to appraise and utilise it. But now more than ever (and in telecommunications more than anything) it takes specialists, in electronics and electrical engineering, to comprehend the intricacies, if not the implications, of the astonishingly broad and complex range of telecommunications "breakthroughs" in the 100 years since Alexander Graham Bell spoke seven words to his assistant on the first telephone.

Telecommunications is widely recognised in Britain as lying firmly within the public domain, being the nuts and bolts of a public service provided by the Post Office, a nationalised undertaking. The Post Office itself acknowledges this; so too does the government. And most people in Britain expect it. Yet the reality looks rather different, for the boundaries of the telecommunications industry are far from coincident with those of the British economy's public sector.

The Post Office, formerly the GPO but made a corporation in 1969, has long had a monopoly of the country's telecommunications *services*. The assets of the system it runs, which is the third largest in the world, are valued at over £4000 million; the Post Office is the country's biggest employer, with almost 250,000 of its 430,000 employees in telecommunications; investment in the year 1974-75 totalled £788 millions, of which 76% (about £600 millions) went on growth and the rest to maintaining and improving the existing system. The figures are enormous; but they form only part of the UK telecommunications picture.

Quite as important is the Post Office's position regarding procurement. In supplies it has nothing like a monopoly. Indeed, just last year, the Post Office produced a document containing recommendations on whether it should manufacture "in-house" more of the substantial amounts of equipment, worth hundreds of millions of pounds, which its existing suppliers produced for it. The question highlighted the network of links between the Post Office, a public corporation, and its main suppliers, all private enterprises with famous names—Plessey, GEC, Pye TMC and Standard Telephones and Cables (STC). A few months later, the nature of those links was further highlighted by announcements from all of these companies of impending mass redundancies as a consequence of a cut-back by the Post Office in its projected orders for telephone exchanges, transmission equipment and telephone sets.

The reasons behind this upset were also revealing. They related, at least in part, to a 30% reduction in the Post Office's estimate on orders for 1975-76 and 1976-77 compared to its estimate a year earlier; this reduction was in turn a reflection of declining demand in the face of rising costs which the Post Office found itself obliged to pass on under the UK government's rediscovered "profitability" regime for the public sector. But the need for redundancies would have arisen anyway, since it was also the result of the design and labour-saving manufacture of electronic exchanges being introduced to replace more conventional electromechanical exchanges and so provide the basis for a massive modernisation programme for the UK telecommunications system.

Reactions to these redundancies also showed the sort of economic factors at work, but revealed political ones too, for the additional unemployment at a time of deepening recession naturally evoked deep emotions. Calls for the introduction of the electronic exchanges to be postponed and for old order levels to be restored, if successful, would have given the Post Office too many of the wrong exchanges which it could not really afford anyway (unless it passed the costs on).

Research and development

That new technologies as well as the plight of the economy are responsible for these cut-backs is just one indication of the central role which research and development plays in British telecommunications. There are others, of course, but a glance at the Post Office's books is sufficient to confirm its importance. In 1974-75, expenditure by the Post Office on research and development climbed fully 30% in value to £28.9 millions. Even more striking, about 93% of this (£27.1 million) was devoted to telecommunications, divided as follows: 59% (£16.1 millions) on "new systems", 25% (£6.8 millions) on "existing systems and related advisory services", and the rest (£4.2 millions) on exploratory research.

A Science Research Council publication released in 1974, surveying current research activities in the UK, says Post Office research and development then involved "some 3,500 staff, of whom about 1,000 are qualified scientists or engineers". The Research Department, now located at Martlesham, the new research centre opened last year, conducts exploratory research into new systems and services as well as the technology, materials and devices to back them up; the Development Department elaborates this work, which focuses chiefly on "switching" (exchanges) and transmission.

Important as it is, however, the Post Office's teams are not the only ones in operation even amongst the public corporations. Work is also done by both broadcasting networks (the BBC and the IBA) and by British Rail. Furthermore, there is the mostly-secret Ministry of Defence activity, through the Signals Research and Development Establishment, the Admiralty Surface Weapon Establishment and the Royal Aircraft Establishment; and the SRC has its own Appleton Laboratory. Each of the Post Office's main suppliers—major companies all—also has sizeable research facilities, on a scale similar to that of the Signals Research and Development Establishment. The work done in these is, the SRC says, undoubtedly influenced by the Post Office, because it is their major customer; between them, in fact, they share several hundred million pounds worth of Post Office business annually. Finally, there are the universities and polytechnics, whose work involved (in 1972-73) 526 people and an expenditure of almost £350,000, the greatest amount coming from the public sector.

The research net is thus spread fairly wide. But what is caught in it? Practically everything conceivable and more besides, if such breakdowns by establishment as are available are anything to go by. While the jaundiced eye might cynically view those research efforts which are made glamorous by publicity-conscious promoters as mere attempts at self-justification, it would probably still focus on the extensively-publicised telecommunications work of the Post Office; after all, the telephone service remains the most direct contact with the world of telecommunications for the large majority of people.

Take the humble telephone set itself. In concept the instrument has changed little: but in a new microphone, sound generates an electric signal from a plastic diaphram with a built-in charge which is boosted by a transistor amplifier. Do the problems of general application, which are large, make such time, money and effort worthwhile? Professor Merriman, Senior Director (Development) with Post Office Telecommunications, has explained that the benefit of such devices lies "in their implied potential for modifying the balance of investment" between telephone sets and their lines to the local exchange. Similar economic arguments are deployed regarding development of the lines themselves. But the chances of reducing costs, without a "fundamental" change in technology, are, he has argued, "reaching an asymptotic limit".

Transmission and exchanges

It is precisely such a change which offers the possibility of transforming the main transmission systems, for here research and development has progressed enormously, particularly in the last ten years. Two entirely new forms of transmission system are being developed which virtually outdate the now-customary coaxial cable and microwave systems.

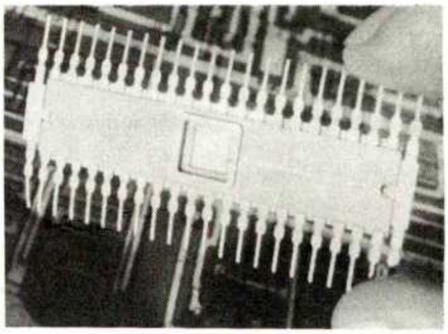
One uses "optical fibres" and transmits signals in the form of light pulses down extremely thin strands of glass fibre. They can carry more messages further and more cheaply with fewer amplifiers than conventional cables. The other uses "waveguides", and is described by the Post Office as "a kind of piped microwave radio"; in it, signals are borne on waveforms down a copper pipe. In both cases digital transmission is involved, where signals are sent as a code of *pulses*, obtained and retranslated through what is known as pulse code modulation, rather than in the customary analogue form, where electrical *waves* corresponding to sound need greater boosting.

But perhaps most attention, certainly in the case of the Post Office supliers, is being devoted to "switching", or exchanges. The key electronic development which is outdating electromechanical arrangements is the integrated circuit, or "chip", hundreds of which are no larger than a pin's head: made of semiconductor, these will replace the large electromagnetic relays; linked to form "processors", they could control central as well as local exchanges. The TXE2 is smaller and uses more basic technology than the TXE4, the electronic exchange developed for large local exchanges of up to 40,000 lines. For the suppliers they offer tremendous export potential, particularly with respect to countries establishing whole new telephone systems.

Switching now represents something like a third of the Post Office's overall investment and is probably the most substantial element of the telecommunications industry's annual production. With the refinement of the electronic exchange, the development path for Britain's telephone system seems fairly clear for the rest of the decade. But it is the result of decisions taken over a period of some 15 years. Moreover, further developments in switching technology are anticipated, and soon. *Their* application demands foresight too; but it also demands consideration of the precise manner in which they will fit in to technological changes in signals and transmission. That means one thing if costs are to go on being reduced in the 1980s: as Merriman has put it, future switching technology "inescapably must be conceived in the context of total systems development and as part of the progressive move towards all-digital, microelectronic, software-controlled systems".

Which introduces what the Post Office calls its "System X", the catchword it proudly uses for overall modernisation of Britain's telephone system. The catchword is necessary: any explanation of it descends quickly into the jargon of systems analysis. The basic idea seems to be that a comprehensive strategy laid now by the Post Office and the industry for the future will, over the years as it is introduced on a larger and larger scale, plan and coordinate the direction of research at all levels, anticipate developments in advance which might in turn be incorporated into the strategy, and generally ensure balanced development and progress.

If the money is there. Thus far, as the figures themselves show, telecommunications has not had it all that bad: few other high technology sectors can point to coherent plans for the 1980s so confidently. And they do not relate only to the telephone system: the list of services now or soon to be provided includes radiopaging (the doctors' "bleepers"), Viewdata (up-to-the-minute information on the telly at the push of a button), Confravision and Viewphone (long-distance conferences between individuals who can see each other and exchange papers) and Fax, or facsimile transmission (a telex-photocopy combination). Even within the realm of telephony itself, improved international dialling and telex facilities, additional use of radio and satellites, automatic dialling devices, push button phones—all are held out, and the list goes on. Fourteen years after Telstar, in fact, space technology still represents a new frontier, and the Post Office has a share in a total of seven satellites.



Integrated circuit for electronic exchange

In Britain, telephones have until recently proved an enormous growth area, and even last year's downturn was then regarded by the Post Office as purely temporary. There are now around 21 million telephones in the whole country. The Post Office argues that real costs to the consumer have fallen over the years, and have done so because of foresight over technological developments in transmission and exchanges. The experts, in telecommunications as in other comparable fields, wish to see the progress made thus far continue at the same if not an increased pace—partly, the cynics would say, to keep themselves in business, though also to supply a continuing demand at a reasonable cost. But projections for the hopefully rosier future can be wrong in an inflationary era. Moreover, the pace of well-financed development being what it is, if demand for various services does grow, even System X could be outdated by the time it is fully installed.

It is precisely this sort of consideration which highlights what many people regard as the real dilemma of telecommunications in Britain 100 years after the telephone: the inevitable duality of a rapid growth in telecommunications knowledge, on the one hand, and, on the other, the lengthy periods needed for appraisal and implementation. Decisions on the direction of research, on appreciation of results and on their application—the argument runs—consume inordinate amounts of time which the additional element of public accountability tends to lengthen still further. It is an argument used to press for greater competition in telecommunications services and to encourage less duplication of research. Others would like to see less research altogether and more grist to the consuming public's mill.

The end result is a mixture of efficiency and inefficiency, profit and loss, bitterness and sweetness: less something Britain can afford than something she cannot afford to be without—and a far cry from those days over a hundred years ago when those who could conceive the idea of transmitting a voice over wires nevertheless thought it would be of little practical value. ☐

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Making the annual pilgrimage

Seven years since it last graced Boston with its presence, the American Association for the Advancement of Science (AAAS) returned there a fortnight ago for its 142nd annual meeting. Roger Woodham reports on a heady week during which some Americans admired their national science effort—and others took it to task

MINORITIES in science, the Viking lander mission to Mars, the inevitable questions surrounding energy policy in general and nuclear power in particular, the views of organisations like Science for the People on everything from genetic manipulation to the busing of schoolchildren . . . this is a selection of topics which is at once typical and atypical of the Boston AAAS meeting. Atypical, because few if any of the 5,000-odd attending the meeting will have found it possible to draw all its diverse threads together; but typical, because it represents in a broad-brush fashion the tenor of the whole meeting. Some impressions:

- Allied to the whole question of opportunities for minority groups in science —consideration of which included papers like "Spanish-surname Americans in the sciences"—was the matter of women in science, or rather the lack of them. This was the subject of an unusual morning session on Great Women in Science, attended as might or might not be expected by 150 women and 10 men. The message was clear—either, like Margaret Burbidge of the University of California (who has recently been voted Woman of the Year in California), they had been actively encouraged from an early age, or, like Mildred Dresselhaus of MIT (one of three women members of the National Academy of Engineering), they had simply "stumbled into science and engineering".

When it comes to recognition of female scientists, it transpires that the National Academy of Sciences only does slightly better than its engineering counterpart. It has 21 women members at present, out of a total of 1,144, and has only ever had 26. But it can claim to have done better than the Royal Society of London in electing its first woman member in 1925 rather than 1948 Equal recognition, the meeting concluded, is as much (if not more) of a problem for women as equality of opportunity.

- One recurrent theme was concerned with the tying together of federal government decisions on tax and economic matters, research and development, and regulatory policy. Dr William D. Carey (Executive Officer of the AAAS) contended that the burgeoning of regulations involving industry had led directly to general caution on the part of innovative industrial concerns and the handing down of cautionary decisions. Ninety per cent of industrial research and development is defensive, not imaginative, he declared.

Taking a broadly similar line, Dr Derek C. Bok, President of Harvard University, made remarks that will be echoed by his counterparts on the other side of the Atlantic. He complained that "the government has felt constrained to make painful choices about research priorities; choices that demonstrate the power of the purse to control the growth and shape of the universities". In times of money shortage, he went on, "the needs of institutions of special quality are readily dismissed as elitist and unnecessary". Crash programmes for this or that are all very well, but by focusing attention so heavily on immediate national problems, public officials run the risk of restricting "advances in fundamental knowledge that will leave us ill-prepared for the crises yet to come". Echoing Dr Carey, Dr Bok said that declining funds threatened the most novel risk-taking forms of science.

- No fewer than fifteen individuals directly involved in the Viking Mars programme—and one Congressman, Don Fuqua, known to be well disposed towards space science—contributed to a day-long seminar that not only mapped out what the mission hopes to achieve, but reflected, quite soberly, on what may happen next.

Viking, or rather the Vikings, for there were two identical spacecraft launched within a few weeks of each other in October last year, go into orbit around Mars on June 19 and August 7. And as Dr James S. Martin, the Viking project manager, put it, there's no flexibility in that date, whereas the patriotic notion of landing Viking 1 on Mars on July 4, at a place in the Martian Northern Hemisphere appropriately called Chryse ("hope"), could be scrapped if the information sent back from Mars orbit suggested that some other time or place for the landing would be appropriate. Viking 2 is scheduled to land on September 4 (in a different place) but again the Viking 1 lander and the part of it left in orbit would be helping to update Viking 2's final plans.

Everyone seemed to be agreed that the future for the space sciences would in large measure be shaped by the results that come out of the Viking project—not only the photography of the landscape, by cameras capable of a resolution of 2 to 4 millimetres, but also the outcome of scooping up Martian soil and feeding it on a "rich organic soup", among other things, to see if there are any signs of life.

If there were, that might also be the signal for renewed activity in the search for extraterrestrial intelligence—another seminar topic which saw AAAS participants packed in the aisles. The idea of spending $10,000 million on Project Cyclops, a giant 'listening' programme, is one that is pretty well buried, but more modest schemes to carry on and expand the listening work of the Arecibo radio telescope would undoubtedly blossom if Viking struck lucky.

Assuming some middle-of-the-road outcome for the Viking project—which certainly means no photographs of footprints—and bearing in mind the constraints of the NASA report *Outlook for Space,* which lays down those classes of project that should be attempted—what alternative ways ahead are there? More Vikings of the same kind, Vikings that can be mobile on the Martian surface, Vikings that drop penetrometers from orbit (instrument packages capable of burying themselves hundreds of metres down in the right conditions and sending back data such as temperatures), Vikings that can return a shovelful of Martian soil to Earth—all these are possibilities, as is further collaboration with the Soviet Union. But what everyone seems to be ruling out is landing a man on Mars, at least before the year 2000. That would undoubtedly violate NASA's guidelines for space research in the last twenty years of the twentieth century.

- Science for the People—a radical group that can see very little right in American science, it seems—has almost made it into the Establishment of the AAAS. During the past five years or so it has had a tolerate–hate relationship with the association at annual meetings, sometimes involving the police; this year it had its seminars formally included in the AAAS programme. Science for the People will snipe at anything—including Boston's school busing problem and the cost of electricity—but at the Boston meeting it was mainly addressing itself to topics like cancer research The group asked, in one of its sessions, for example: "Why has so little effort been devoted to the problem of preventing cancer caused by occupational and environmental agents, when it is widely recognised that 80 to 90% [*sic*] of all cancer is caused this way?" □

THE GAMBIA

More the people than the place

Dr R. S. Bray, Director of the Medical Research Council laboratories in The Gambia, looks at life and work there.

THE 1975 balance sheet for medical research in The Gambia shows a reasonably healthy credit. Not everyone wants to do their medical research in a tiny republic stuck out in the savannah of West Africa, but there are compensations. The research is directly about people: organisms and medical phenomena are in people not guinea pigs, and epidemiology figures large. For this you need not merely people, you need nice friendly people. and an understanding population helps to make this the Kingdom of Heaven to an epidemiologist.

The Gambia's people have a willing belief in the delights and benefits of medical research and a considerable dignity to abash the over-assertive. When it came to importing a South African worker to do some haematological investigations, it was with some misgivings that the Ministry of Health was approached about his entry. But—"Bring him in," they said, "we'll show him how nice we are". And they did: he came back the following year and is now thinking of teaching them Rugby!

There are some drawbacks, of course. The rainy season can be tiresome, though there's lots of lovely organisms rife. Distinct seasons do make epidemiology interesting, but it's hard to work full-time on parasites which insist on disappearing by February and not reappearing until July. And of course we are remote. The cut and thrust of one's peers is sorely missed—each researcher tends to be working on a sufficiently different subject to be able to make confident statements based on shaky premises with never a demur heard.

Efforts continue to be made to get onto good terms with The Gambia's neighbours. Visits to Dakar are regular for such essentials as liquid nitrogen and frog's legs. But efforts to revive some institutional form of anglophone West African co-operation in medical research move slowly. The giant to the south—Nigeria—looms over all calculations. They have their own organisations, and the knowledge that they will foot the larger part of the bill for regional co-operative efforts doesn't impel them precipitously into such endeavours. But some concrete results have emerged. A collaborative investigation on malaria and the oral contraceptive pill was initiated between the Komfo Anokye Hospital, Kumasi, Ghana and the MRC, Gambia with IPPF acting as midwife. Equipment has been another productive source of co-operation. It isn't worth £15,000 (for a cobalt 60 source) to irradiate some malaria infected mosquitoes occasionally, so they are taken to Accra where the Ghana Atomic Energy Commission's machine is used with their enthusiastic co-operation.

Malaria figures large as always in medical research. It is still uncontrolled in Africa though the easy availability of antimalarial drugs and domestic sprays is having an effect in and around cities. The sexual forms of the malaria parasite are being studied to see how and when it arises. The major gaps in the knowledge of African malaria are the reasons for the slow and gradual acquisition of immunity, but techniques for exploration of this phenomenon are hard to come by. Work is going on to investigate malaria in the first six months of life and in pregnant women, where variations in immune status may offer some clues. Vaccination is on the horizon, and work continues on the techniques necessary for the collection of suitable antigen Work is also done on filariasis and the onset of elephantiasis, schistosomiasis and the longevity of the worm in the face of acquired immunity, the effect of various diseases on cellular immune processes, malnutrition and the contribution of disease to the onset of marasmus, the manner in which female mosquitoes find their hosts, the contribution of mycotoxins to primary hepatoma and on the susceptibility of N'Dama cattle to trypanosomiasis.

And of course there is the grandest tropical health study of them all—Ian McGregor's 26-year-old survey of 4 rural communities in West Kiang district. This unique work brings together 26 years of morbidity and mortality data, growth statistics, endemic and epidemic disease information, serological results and medical genetics. When eventually collected and collated it will form a standard of reference for medical demography and for medical genetics unsurpassed in Africa. Already the information spun off has been of enormous importance.

Always looming, however, is the fact that 4 to 5 of every 10 children born in Black Africa die before adolescence. When projects are designed, this fact can encourage, even justify—for if an experiment works which brings the disappearance of this disease forward one month, 100,000 lives might be saved. A sobering thought. □

IN BRIEF

JET lag

EEC efforts to take the Joint European Torus (JET) fusion reactor from the design to the construction and experimental stage were still stuck firmly in the political mud last week when community ministers failed to agree on a site for the project after a 16-hour tussle in Brussels. There was agreement on building and financing the project, but national opposition to an earlier Commission finding in favour of the site at the Community's joint research centre at Ispra, northern Italy, forced an impasse which the ministers finally sought to resolve by establishing a new committee; this will investigate the relative technical advantages of the contending sites and decide whether experience in fusion research and plasma physics is relevant in choosing between them. Ministers are due to meet again in mid-June, over six months after the next five-year stage of the £96-million EEC fusion research programme should have begun

Nuclear trade measures

In Washington, a Senate sub-committee on arms control heard last week that the "Group of Seven" nuclear exporting nations—Britain, Canada, France, Japan, the Soviet Union, the United States, and West Germany—have agreed still-secret measures to extend safeguards against the misuse of exported nuclear technology. Some clue to the nature of the pact comes from new US Government principles which require foreign recipients of nuclear facilities, equipment and materials to comply with the internationally accepted safeguards already laid down by the International Atomic Energy Agency (IAEA), and to demand identical assurances from any country subsequently handed similar technology. Recipients will also be restricted from using imported facilities for explosive purposes, whether peaceful or not, as a result of the new US policy.

Serendipity in science?

"There is nothing more practical than a good theory", said Mr Brezhnev, addressing the Twenty-Fifth Congress of the Communist Party of the Soviet Union last week. These words, reiterated by Academician Anatolii P. Aleksandrov, President of the Soviet Academy of Sciences, were apparently intended to justify expenditure on basic research in a Five Year Plan which urges that science should be made cost-effective and more closely linked to production. Citing examples ranging from nuclear physics and MHD-generators to cytology and genetics, Aleksandrov stated that basic research "radically changes technology, leads to the appearance of new materials, and opens up possibilities of using new, often unexpected phenomena, which have no connection with the original field of research".

Dragon reprieve?

The death-throes of the 15-year old Dragon high temperature reactor project at Winfrith in the UK may last longer than expected if a proposal from the European Commission in Brussels is approved. The Commission wants to see the team kept together until the end of the year, mainly to allow the results of the whole project to be evaluated, but also, in turn, to leave the way open for any possible but unlikely resuscitation. The proposed maintenance would cost about £1½ million.

BNFL complication

As British Nuclear Fuels Ltd awaits cabinet approval for its controversial Japanese nuclear processing deal, there are reports of French moves to break into the contract Britain, being a member of the reprocessing group set up with France and Germany known as United Reprocessors Ltd, is obliged to discuss the French bid for a possible 50% share in the contract, or risk competing with France for the entire deal, worth up to £600 millions over the decade following 1980.

Dumping decision

Whatever the outcome of the BNFL negotiations, Britain is to continue dumping low activity material in the deep ocean. In a House of Commons written reply last week, the UK Energy Secretary Anthony Wedgwood Benn stated that desirable disposal rates of the "low activity plutonium contaminated solid radioactive waste", stored mostly at Windscale and Drigg in Cumbria, are well within International Atomic Energy Agency safety limits. They do, however, exceed the dumping levels arranged by the Nuclear Energy Agency of the Organisation for Economic Cooperation and Development, and Britain is to push for higher limits.

Angling right

KENNETH MELLANBY

SOME members of the Council of the Royal Society for the Prevention of Cruelty to Animals (RSPCA), following their success in persuading the society to oppose fox hunting, are trying to engineer the acceptance of a similar policy towards angling. They say it is cruel and should be banned. If they are successful, the conservation of our environment, and in particular of our rivers, could suffer great damage.

Angling is by far the most popular outdoor sport in Britain, having as many as three million adherents. On an average weekend during the fishing season, there are likely to be as many as five individuals sitting patiently by our rivers and canals waiting for a fish to bite for every one spectator attending a professional football match. Although some members of angling clubs from the midlands may lighten the tedium of their coach journey home from the dykes of East Anglia by song lubricated with bottled beer, this seldom if ever leads to the sort of vandalism apparently inseparable from watching some of our more notorious soccer teams The unobtrusive behaviour of fishermen generally is the reason why others are so surprised to learn of their numbers.

However, anglers can be aroused to effective action when their waters are polluted. Although salmon and trout can flourish only in the cleanest streams, even so-called "coarse" fish (which supply the bulk of the sport in England) are delicate indicators of pollution. There have been thousands of occasions when the first warning that some unauthorised and toxic effluent has been discharged into a river has been given by a fisherman who has spotted dead or dying fish which might never have been noticed by the river authorities. The Anglers' Cooperative has been effective in taking legal action to stop such practices Of course anglers can only operate in rivers pure enough to contain fish, and so our filthiest waters do not receive their attention. This is perhaps one reason for the slowness with which they are cleaned up. But once a river is clean enough to be invaded by fish, it is the angler who plays the greatest part in safeguarding it and who may be responsible for further improvements.

Anglers not only outnumber other sportsmen; they also greatly outnumber all our organised environmental and wild-life conservationists. Angling is, furthermore, a sport with adherents from all social classes, including those unlikely to be involved in the more elitist forms of conservation. Yet, as I have indicated, anglers are really *effective* conservationists. It would thus be to their mutual benefit if the two groups could get together to cooperate whenever possible. If bodies like County Conservation and Naturalists' Trusts could recruit a proportion of the anglers operating in their area, they could often quadruple their numbers and end the criticism that they are only a bunch of middle class protectionists working for their own selfish ends. The conservation bodies could help the anglers, for instance, by making waters in suitable reserves available for fishing, and by preventing bodies like the RSPCA (many of whose members are the self-same middle class conservationists) from endorsing policies likely to damage the countryside.

Presumably the move to ban angling is an attempt to benefit the fish. It might prevent a number of fish from being hooked, an experience which can hardly be pleasant, though there is disagreement about the extent to which fish and other cold-blooded animals experience what we describe as "pain". However, without the vigilance of the anglers, fish kills would almost certainly become commoner, and fish might be completely exterminated from many of our waters. It is not unreasonable to suggest that the experience of a fish, choking to death in unnecessarily deoxygenated streams, or writhing in apparent agony after exposure to poisoned effluents, could involve greater cruelty than that arising from normal fishing practices.

Today there is such pressure on our countryside and on its wildlife that all forces need to be mobilised to protect the environment. Freshwater is the habitat most endangered by pollution. It is to be hoped that those members of the RSPCA who are so strongly opposed to fishing will come to realise what would be the eventual consequence of their present policy. Fortunately they are more likely to destroy their own society than to stop a sport which has such widespread support from all sections of the community, and which contributes so much to preserving our environment.

correspondence

Alternative refrigerants

SIR,—We have carried out a survey of possible substitute refrigerants that could be used in the event that it becomes necessary to ban the use of refrigerant-12, CF_2Cl_2. Very few compounds meet the requirements of being nontoxic and nonflammable, having long-term stability in a refrigeration system and a short residence time in the lower atmosphere. A good alternative to R-12 must have a normal boiling point near −30 °C. We have considered all compounds known to boil between −45 and −15 °C. The list includes approximately 80 compounds of which all but seven can be immediately ruled out as good alternatives. The remaining candidates include CF_3I (b.p.= −23 °C), C_3F_8 (b.p.= −36.7 °C), *c*–C_3F_6 (b.p.= −33 °C), CF_3CH_2F (b.p.= −26.5 °C), *c*–C_3OF_6 (b.p.= −29 to −30 °C), $CF_3OC_2F_5$ (b.p = −23.3 °C), and CHF_2Cl (b.p.= −40.8 °C).

Of these compounds CF_3I may have inadequate long-term thermal stability, decomposing to the products C_2F_6 and I_2. We are uncertain as to the flammability of CF_3CH_2F, and it would be very difficult to prepare this partially fluorinated hydrocarbon in good yield. Perfluoroproprane, C_3F_8, appears to be an ideal refrigerant. We are concerned, however, that this compound would have a much longer atmospheric residence time than R-12 and thus build up to be photolyzed at much longer wave-atmosphere. This would be undesirable since it is not possible to predict beforehand all possible environmental effects of a contaminant. Because of the strain energy in perfluorocyclopropane, *c*–C_3F_6, we expect that this compound to be photolysed at much longer wavelengths than C_3F_6 and thus have a shorter atmospheric residence time. Also, the boiling point of this compound is nearer to that of R-12 and for this reason would be a better substitute. The cyclic ether, perfluoropropylene oxide, and perfluoromethylethyl ether appear to be good substitutes. Presumably, these compounds would rapidly decompose once released into the atmosphere. We are concerned that these compounds may have anaesthetic properties and that they may lack long-term stability in the presence of trace amounts of water. Refrigerant-22, CHF_2Cl, is already used in a wide range of applications. However, its boiling point is much lower than that of R-12 so that its vapor pressure is higher, and thus requires much sturdier and therefore much more expensive refrigeration hardware.

Our civilisation can function without aerosol cans, but not without refrigeration Except for R-22, any of the alternatives we have suggested would require five to ten years to be produced at the required level. Any decision to ban the use of R-12 should take into account these facts

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Carbon dioxide reduction

SIR,—As is well-known, the oil crisis raises three distinct technical problems: overall shortage of fuel, shortage of suitable portable fuel (now normally hydrocarbons), and shortage of raw material for organic chemical industry.

Several possible solutions have been advanced for the overall fuel shortage and, if they prove adequate, this should cease to be a problem. If not, we are in a more difficult situation than most of us yet realise.

It has been proposed that the use of hydrogen, readily available with sufficient power, may be an answer to the second problem. For the third no long term answer has been advanced; the use of coal will provide a temporary answer after oil is exhausted, and coal can also be used to produce portable hydrocarbon fuel.

I have already suggested (*Nature*, **251**, 469) that it is worth looking again at the production of furfural from agricultural waste as a partial, but renewable, answer to the second and third problems.

I do not myself believe that the technology of the liquid hydrogen economy will prove practical on the wide scale, and it seems to me that only organic compounds, though not necessarily hydrocarbons, can provide an answer to the need for a portable fuel.

Thus an answer to both the second and third problems may need a large scale, renewable source of organic compounds. The only inexhaustible source of carbon is carbon dioxide. Given an adequate supply of hydrogen, it is possible to reduce carbon dioxide as is normally done with carbon monoxide in the Fischer Tropsch reaction. It is the purpose of this letter to draw attention to this possibility, and to suggest that the time for basic investigation of this reaction is now, before the need arises.

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Science and industry

SIR,—The urgent need for 'Harnessing Science and Industry' in Israel (February 12, page 442) must also apply in the UK. Indeed, discussion is urgently required as to how better use might be made of British scientific innovation and talent, and your leading article (January 29) has done something in this direction by touching upon the possibilities of encouraging more scientists into industry.

But the problem is both deep-seated and urgent. There was a move recently to focus attention on this problem by establishing an exhibit (and perhaps a study centre) in buildings surviving from one of the earliest science-based industries in this country—that is, on the site of the historic Perkin and Sons dyeworks at Greenford Green, Middlesex, a mid-nineteenth century enterprise with an enviable record for turning the results of the latest scientific research into technological and commercial success. Ironically, this move failed in January of this year with the deliberate demolition of the buildings of interest due to a lack of appreciation of scientific and technological matters by businessmen and civil-service administrators.

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IQ tests and majority groups

SIR,— In common usage, and by statute for elections in the USA, "majority" is a number of excess of half the total. In a total with three or more subgroups, the largest is a "plurality". A plurality may also be, but need not be, a majority I suggest that in Dr Harrington's very valuable study (December 25, page 708), since none of the populations contained a majority, the largest group in each population was a plurality. His results seem to indicate that the more numerous, or principal, groups are favoured by current testing procedures; a most important finding indeed

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news and views

Social insects and the evolution of altruism

from John Krebs and Robert M. May

It is an astonishing fact that many professional biologists still do not understand the essence of Darwinian natural selection. Many people still think, and write, that adaptations evolve by natural selection because they are 'good for the species as a whole'. This sort of statement can be found in such influential writings as Konrad Lorenz *'On Aggression'* and the Nuffield Sixth Form Teachers' Guide. Natural selection is a matter of differential survival and reproduction of individuals (or to be precise of genes), not of species. Adaptations evolve because they enable genes in individuals to out-do genes in their conspecifics, and natural selection relentlessly pursues this course without regard to the survival of the species or population as a whole. For many purposes it does not matter whether one appreciates this distinction, but in analysing the evolution of social behaviour it is crucial, so it is not surprising that some of the most important developments in understanding natural selection have come from this field.

The central theoretical issue in the evolution of social behaviour is the problem of altruism. In biological terms, an altruistic act is one which decreases the fitness (survival and reproduction) of the actor, while increasing the fitness of another individual (the recipient). If such genetically altruistic behaviour occurred in nature, it would undermine the very core of Darwinian natural selection, which is why the problem is so interesting.

Charles Darwin himself remarked in the *Origin of Species* that the apparent altruism demonstrated by the social insects posed 'the one special difficulty which at first appeared to me insuperable, and actually fatal to my whole theory'. He then went on to give reasons why natural selection may sometimes operate at the level of family groups rather than individuals, in effect anticipating the idea of 'kin selection' which plays such an important part in modern ideas of the evolution of social behaviour in animals.

Some types of apparent altruism such as parental behaviour are easily understood. A parent bestows care on its young, often at considerable cost to itself, but of course the young represent the parents' gene contribution to the next generation and natural selection by definition favours individuals who maximise their gene contribution to future generations. But children are not the only bearers of a parent's genes: brothers, sisters, cousins and so on all share a considerable proportion of their genes by common descent. So the same reasoning that applies to parents and their children applies to all kin. W. D. Hamilton, in two seminal papers (*J. theor. Biol.*, **7**, 1, 17; 1964), developed the concept of inclusive fitness—the idea that in order to estimate the fitness of an individual it is necessary to take into account the survival and reproduction of kin. In practical terms, you might make a genetic profit by helping your brother to have an extra child, even at some cost to yourself, because your brother's child shares a quarter of your genes by direct descent. The general conditions favouring this type of 'kin altruism' can be stated precisely.

Your kin share a certain proportion of your genes, for example full siblings have a probability of 0.5 of sharing any particular gene by descent; nephews and uncles 0.25, and first cousins 0.125. This probability of sharing genes is referred to as the coefficient of relationship r; more formally it is defined as the conditional probability that individual B has a particular gene given that individual A has it. An altruistic act will benefit (in genetic terms) the actor as long as the benefit to the recipient is greater than the cost to the actor by a factor equal to $1/r$.

In the case of full siblings if you sacrifice your life for your siblings, more than two must survive as a result of your death for the 'altruistic' sacrifice to increase your fitness. Any apparently altruistic behaviour which can be accounted for within this framework is genetically selfish and therefore consistent with Darwinian theory, for since gene types are the basic stuff of evolution, it is inclusive fitness, not individual fitness that is maximised by natural selection.

This important concept of inclusive fitness was used by Hamilton to explain the evolution of the most extreme altruistic behaviour in the animal kingdom, that of worker castes in the social Hymenoptera (bees, wasps and ants). In these social insects the reproductively competent queen produces sterile worker females, whose task is to spend their life rearing the other fertile male and female offspring of the queen. Worker behaviour superficially appears the ultimate in disadvantageous behaviour from the point of view of the individual perpetuating its genes. But, because in the Hymenoptera the males are haploid and the females diploid, the worker female is in fact more closely related to her sisters than to her own hypothetical offspring. Daughters contain a full set of their father's genes, and so sisters related through both parents have a higher degree of relationship to each other ($r=0.75$) than to their own children ($r=0.5$). Conversely, a male is more closely related to his daughter ($r=1$) than to his siblings ($r=0.5$). Females are related to their brothers with $r=0.25$.

So the daughters of a queen who are faced with the choice of rearing their own daughters or staying at home to rear younger sisters should prefer the latter course (which is possible in the social Hymenoptera because the generations overlap) because by rearing sisters, a female produces more of her genes in the next generation than by rearing the same number of daughters.

The haploid/diploid separation of the sexes in the Hymenoptera explains why sterile worker castes have evolved at least eleven times in this one group but only once (in the termites) in the whole of the rest of the class Insecta, in which both sexes are diploid, although the Hymenoptera comprise only 6% of all insects. A particularly telling point is that this altruism is sex-limited: only females are workers and all males are reproductive.

In an exciting new paper, Trivers and Hare (*Science*, **191**, 249; 1976) have developed Hamilton's theory further and produced dramatic evidence to support its predictions. They begin by remarking that although this asymmetry in the way Hymenopteran individuals are related to one another predisposes daughters towards the evolution of eusocial behaviour (defined as that combination of co-operative brood care, division of the group into reproductive and sterile castes, and overlap of at least two generations, which characterises the 'truly' social insects), it does not compel it. Trivers and Hare have now clarified the precise circumstances in which asymmetric degrees of relatedness can lead to the evolution of eusociality and have generated several predictions which can be tested quantitatively.

Whereas a female worker is more closely related to her sisters than to her hypothetical daughters, this is not so for her brothers, with whom she has a r of only 0.25 (as Hamilton well realised) If a worker invested an equal amount of effort into rearing brothers and sisters her average r to the siblings she rears would be 0.5, no better than she would do by having her own children. Hence, if the workers are to make a genetic profit from their apparently selfless devotion to rearing siblings, they must put more effort into rearing reproductive sisters than into brothers. The appropriate extra effort follows from the r values above and is a 3:1 preference for sisters over brothers in the sexual brood (the ratio measured, for example, in dry weight). With this ratio, the gene which the worker shares with the male is three times as likely to be present at the foundation of the new colony than the gene which the worker shares with the female, thus cancelling out the initial difference in r between the worker and the male.

On the other hand the queen of the colony, who actually lays the eggs prefers an equal investment in the sexes among her progeny (as proved by R. A. Fisher many years ago as a general rule for all sexually reproducing organisms). So within the colony there is a conflict of interests: the apparently altruistic worker can only pursue the genetically selfish course dictated by natural selection if she devotes three times as much effort to reproductive sisters as to brothers, whereas the queen can only maximise her fitness by equal investment.

Therefore, when a mother queen lays all the eggs and the worker caste is completely in control of rearing the larvae, the ratio of investment should approximate 3 : 1. On the other hand, if the queen has control over the ratio of investment, it should remain at 1 : 1. When one or more workers lay eggs the situation is complicated and intermediate. In no circumstances is there any advantage to altruistic behaviour by the haploid males.

Trivers and Hare present good evidence, culled from various sources, for the sex ratios of reproductives ("alates") produced by some 21 ant species in which one queen lays all the eggs (monogynous). Sex ratios and relative weights are obtained by digging up complete nests during the time when alates are present, and from these data an estimate of the relative cost to a colony of a female alate compared to a male can be made. There is a good fit to the predicted 3:1 ratio; the departure from a 1:1 ratio is highly significant ($P<<0.01$).

Various other tests are carried out, although in most cases the data are softer or scarcer, and the points on the regression plots more scattered, than for the monogynous ants. Thus in slave-making ants, the queen's brood is reared not by her own daughters but by slaves (stolen from other species) who have no genetic relationship to the brood. Consequently the queen should achieve her preferred ratio of investment of 1 : 1. Two slave-making species indeed have ratios close to 1 : 1, while three closely related non-slave makers (of the genus *Leptothorax*) adhere to the 3 : 1 ratio. In polygynous ant species, a queen may permit one or more of her fertilised daughters to settle within her nest: large polygynous nests may contain granddaughter or even later generation queens. This makes for a complicated situation, with a tendency for ratios of investment to be biased towards males. Such seems to be the case for seven polygynous species for which data are available. The Hymenoptera also include solitary, non-social bees and wasps. Estimates of the ratios of investment in twelve species of solitary bees and five species of solitary wasps are scattered near 1:1, and none are greater than 2:1. Corresponding ratios for eusocial bees and wasps are hard to come by, because there are time-consuming technical difficulties in distinguishing reproductives from workers. The ratios of investment for five species of social bumblebees (*Bombus*) are scattered in the range 1.2 : 1 to 3 : 1.

Trivers and Hare's paper is of profound importance. It puts the capstone to an edifice that draws together genetic principles and Darwin's theory of natural selection towards explaining the evolution of the social insects. Many other questions now open up. For example, among the most complicated caste structures are those belonging to some of the "leaf-cutter" ant species of the tribe Attini. These ants bring plant material into their nests, where it is used to culture fungi which they eat: very crudely, they may be thought to have accomplished the transition from the "hunter-gatherer" existence of most social insects, to an "agricultural" society. It is surely not coincidental that they possess one of the richest caste systems, and it will be interesting to understand how it came about.

Besides providing direct evidence for Hamilton's kingship theory, Trivers' data are the first to test the outcome of parent-offspring conflict. This type of conflict must be widespread in animals with parental care, because a child is related to itself by a r of 1.0 and to its mother by 0.5, so that the two individuals have different genetic interests dictating their behaviour. Although R. D. Alexander (*Ann. Rev. Ecol. Syst*, **5**) has developed the hypothesis that parents always have the upper hand over their offspring, Trivers' data show the opposite.

As so often happens in the evolution of ideas, it now seems that the social insects, which initially seemed to threaten Darwin's theory, are providing it with one of its most dramatic and quantitative triumphs. □

Organometallic chemistry

from Ronald Mason

'WHAT is the use of a book' thought Alice 'without pictures or conversations' The hundredth issue of the *Journal of Organometallic Chemistry*—a growth stock if ever there were one—is a unique issue in that the editors invited some of the leading research workers to contribute a personal account of their involvement in the field and discuss its development. The result is fascinating—the single picture is of lamentable quality but the conversations go a long way to eroding the old adage about scientists not understanding history (and *vice versa*!)

Organotransition metal chemistry owes much of its present shape and status to the discovery of the π-cyclopentadienyl and *bis*-benzene 'sandwich' compounds in 1951 and the two Nobel Laureates associated with the discovery —Fischer and Wilkinson—provide articles which are interesting in their contrast. Wilkinson's recollections are intensely personal and warming to any advocate of the proposition that science is, above all else, a human activity. Fischer chose to discuss his most recent results on carbyne-complexes so we are deprived of the comparative viewpoint. Retrospectively, one of the more interesting points about the immense literature which has been generated by the original work is that the compounds have provided little direct spin off, most of the present concern in organometallic and low valent complex chemistry concentrating elsewhere, with metal carbonyls, π-alkyl complexes, tertiary-phosphine and -phosphite complexes and σ-bonded compounds providing more scope in synthesis and catalysis; if one accepts this argument, the articles provide for introspection and the prospects for the subject are not easily discernible.

The future for organometallic chemistry, and indeed for chemistry, is stiffly linked to the development of new approaches in synthesis. The synthetic strategies of organometallic chemistry are much less determined and sophisticated than those of organic chemistry but the serendipities of the past twenty years have been signposted and a rational basis for synthesis is emerging. Thus, so far as σ-bonded transition metal organometallics are concerned, the realisation of the difference between their kinetics and thermodynamic stabilities has led several research groups to design stable molecules through precluding, *inter alia,* the β-elimination reaction. The use of metal atom vapours in synthesis is providing species which had wrongly been thought of as thermodynamically unstable and these complexes may see much use as precursor reagents The philosophy of the stabilisation of reactive species such as cyclobutadiene and benzyne is encapsulated in an article by Pettit and extrapolated to metal-carbenes and -carbynes which have much potential as intermediates in synthesis. In the context of the use of metals in organic synthesis, an obvious omission is one which takes no explicit account of the work of Wilke and his colleagues, particularly the recent studies in chiral and heterocyclic ring synthesis.

It will be interesting to see whether, as I believe, there will be a substantial shift soon in emphasis from organo-transition metal chemistry to main group organometallics. Brown, Gilman and, to a lesser extent, Hawthorne help us to anticipate the move but the main reasons for such a change are that, following a lengthy period of establishing the basic methods of synthesis and general properties of Group IVB-organometallics, many novel advances are coming along quickly including the fairly convincing demonstration of the existence of long-sought species such as $Me_2Si{=}CH_2$ and $Me_2Si{=}O$, the easy generation of silylene species, and the discovery of a wide range of novel rearrangement reactions. The upsurge of the use of organosilicon compounds in organic synthesis has reached into industrial processes with trimethylsilyl compounds involved in the manufacture of antibiotics. Eaborn touches lightly on this area in the hundredth issue, particularly with respect to the potential synthetic use of aryl-MR_3 bond cleavage. And one can remember the long standing use of organo-lead and organo-tin compounds A long term future for main group organometallics is assured again by the recent discovery of the biological activity of certain cyclic siloxanes, which have similar properties to the oestrogenic steroids, and of the silatranes whose versatile therapeutic properties include claims for the treatment of baldness! One might go further. parenthetically, and generalise beyond organometallic chemistry: main group chemistry is now ready for the intensive approaches and techniques which have given a maturity to 'd' block chemistry.

It seems a pity that structural aspects of organometallic chemistry are hardly considered explicitly, given the symbiotic relationship of synthesis and, in particular, X-ray crystallography. Our understanding of the metal–carbon bond has changed profoundly, not least because of the insight which the Dewar-Chatt model provided for metal π-complexes It is obviously a matter of personal taste and prejudice but, given his immensely varied and seminal contributions, I would have preferred to see Chatt discussing metal-carbon bonds and the reactivity of low-valent complexes, rather than highlighting the recent advances in metal-dinitrogen chemistry; but that part of his article which deals with the formation of nitrogen-carbon bonds can be assured of concentrated study over the next five years Cotton's article on the dynamic or fluxional behaviour of organometallics in solution substantially remedies the balance but here one wonders if the last milestones have not been passed and that it will be other approaches which will provide a quantitative description of intramolecular potential energy surfaces

A final thought is appropriate on the mechanisms of reactions of organometallic molecules, a topic which is in a primitive state compared with that obtaining in physical organic chemistry. Surely this is an area of mainstream development for it involves study of the reactions of coordinated ligands and the metal-activation of covalent bonds, two matters which lie at the heart of any discussion of homogeneous and heterogeneous catalysis. Stone's contribution reminds us of the tremendous effort that has gone into studies of organometallic and coordination complexes of d^8 and d^{10} metal ions and which have provided a variety of homogeneous catalysts and, for example, a much better understanding of the role of steric effects in reactivity (*vide* the activation of carbon-hydrogen bonds). The chemistry of polynuclear complexes has only just begun in anything like a systematic way and persuasive evidence is already available that some may not only be effective catalysts in their own right but their reactions can provide insight into the spectrum of physical and chemical processes that occur at surfaces of heterogeneous catalysts.

There have been sceptical, well-publicised comments on the maturity of organometallic chemistry which have carried the implication that the field will provide few novelties in the future. This proposition can be debated over all researches in the natural sciences but the prospects of metal ions providing more versatility and control over the reactions of organic molecules seem clearer and more rationally based than those of a decade ago. The two hundredth issue of the *Journal of Organometallic Chemistry* could provide an acid test of this forecast. ☐

Revolutionary SiC fibre

from Robert W. Cahn

G. K. CHESTERTON once wrote a rollicking poem about the Rolling English Road, and commented affectionately on the entertaining journey from somewhere-or-other to Birmingham, by way of Beachy Head. Anyone who knows England knows that it is not sensible to go to Beachy Head if you want to finish up in Birmingham, no matter where you start from. But it was an Englishman who had the crazy notion of making pure carbon (Birmingham) by first making an elaborate polymer (Beachy Head) and then destroying it by heat. Watt's technique of stretching oxidising and pyrolysing polyacrylonitrile to create strong carbon fibre was highly unconventional, but it worked triumphantly.

Now an equally eccentric and equally brilliant Japanese chemist, Seishi Yajima, has found an equally unconventional way to make strong, continuous fibres of silicon carbide. A preliminary summary of his method has been published in *Chemistry Letters* (Japan), (No. 9, 931; 1975).

More detailed publication is on the way, and Yajima (who is head of the Oarai branch of the Research Institute for Iron, Steel and Other Metals of Tohoku University) has also privately circulated an account of his underlying philosophy. He explains that conventional organic chemists have long striven to convert inorganic materials to organic (this goes right back to Wohler's synthesis of urea), but they have an inbuilt resistance to turning back an organic material, attained in complex form by considerable efforts, into an inorganic material of simple structure by heat or chemical treatment 'The organic chemist intrinsically detests a high temperature. He cannot stand the destruction of a complicated structure which has been obtained by painstaking efforts.' Yajima is an unconventional organic chemist who has overcome the phobia of fire.

Fibre-reinforcement for service at high temperatures is fraught with difficulties Carbon fibre is too reactive, alumina is too weak, SiC whiskers are too short and too expensive. Intrinsically, however, SiC has all the required properties, and Yajima set out to make strong, continuous fibre He starts with dimethylchlorosilane, $(CH_3)_2SiCl_2$, reacts it with lithium and a catalyst to form dodecamethylcyclohexane, $[(CH_3)_2Si]_6$, which is polymerised in an autoclave The molecular weight fractions are separated by repeated solvent extraction and a solution of molecular weight ~1,500 is solvent-spun into a fragile, brittle fibre This is then pyrolised in a vacuum at temperatures in the range 800–1,300 °C to turn it into β SiC. Infrared spectroscopy and X-ray diffraction were used (the details are in process of publication) to follow the stages of pyrolysis The best fibres (1,300 °C heat-treatment) have strengths of 300–350 kg mm^{-2}, and elastic moduli of 30 mm^{-2}. Diameters are typically 10–20 μm

The extraordinary feature of the fibres is that although their strength matches that of SiC whiskers, their structure is totally different. Electron microscopy and analysis of X-ray diffraction line profiles both reveal a structure of minute crystallites, 7 nm across, without preferred orientation. This fine structure is crucial if pyrolysis is done at 1,500 °C, giving considerably larger crystallites, the strength falls sharply. Prolonged attempts were made to match this structure by sintering ultrafine SiC powders, but these were never fine enough to achieve high strength. Yajima can only speculate as to the reasons for the remarkable strength associated with the ultrafine morphology

The SiC fibres are extremely resistant to heat and oxidation, and will withstand, undamaged, prolonged exposure under load to a flame at 1,200 °C. By contrast, SiC whiskers lose strength above 600 °C No information is given about the economics of the process, but it appears that the process can be made to work on an industrial scale.

Yajima claims that his work is the first instance of a metal atom being introduced into the skeleton of an organic polymer. This innovation has resulted in an inorganic product which appears at last to open the way to an effective fibre-reinforced composite, SiC-in-metal, which can give effective service at high temperature. ☐

Signals for protein synthesis

from a Correspondent

WHAT features of mRNA primary or secondary structure are responsible for protein synthesis? Possession of a 5′-terminal AUG allows synthetic polynucleotides to function in many initiation assays but initiation codons are by no means unique to initiation sites. In eukaryotic messages, 'cap structures' have recently been shown to aid ribosome binding (Both *et al.*, *Cell*, **6**, 185–195; 1975) but no comparable feature has been found within prokaryotic mRNAs. Here the picture appears to be more complex, and considerable perception on the part of Shine and Dalgarno (*Proc. natn. Acad Sci. U S.A.*, **71**, 1352–1346; 1974) was required to identify what, recent evidence suggests, is indeed a signal for bacterial ribosomes.

Shine and Dalgarno point to a group of residues, mostly purines, found preceding the initiation codon by about 10 bases in the various initiation sites which have been sequenced—this partially conserved sequence could potentially base-pair with the 3′-terminus of 16S rRNA They cite evidence implicating this part of the rRNA molecule in initiation and postulate that the selection of an initiation site is influenced by the potential of any given site to base-pair with 16S rRNA (Shine and Dalgarno, *Nature* **254**, 34–38; 1975). What is lacking in this imaginative hypothesis is any direct evidence that this base-pairing actually takes place, and it is this point to which Steitz and Jakes (*Proc. natn. Acad Sci U.S.A* , **72**, 4734–4738; 1975) have addressed themselves

They make use of the ability of colicin E3 to cleave 16S rRNA 50 bases from its 3′-terminus When *E. coli* ribosomes were allowed to initiate on the ^{32}P-labelled A protein initiation site of R17 phage and were then treated with colicin E3, the detergent SDS released most of the counts as a complex between the labelled initiation site and the 3′-terminal fragment of 16S rRNA. The complex was detected on polyacrylamide gels migrating more slowly than either the free initiation site or the colicin fragment—without colicin, the counts were recovered at 16S on sucrose gradients. The complex is an RNA · RNA duplex since it can be formed by the isolated RNAs, though presumably the extent of its formation is then much lower. The crucial observation is that if the binding of mRNA to ribosomes is inhibited by aurin tricarboxylate, only small amounts of this complex can be detected. Since this inhibitor does not affect the annealing of RNA, the most reasonable conclusion to draw is that binding of ribosomes to the initiation site facilitates the 'Shine–Dalgarno' base-pairing. This approach cannot tell us whether this interaction is occurring during initiation or during the subsequent disruption of the ribosomes Few people would deny, however, that these experiments give substantial support to the hypothesis of Shine and Dalgarno.

If we may accept that base-pairing between the R17 A protein initiation site and the 3′ end of 16S rRNA takes place during initiation, we must examine whether this phenomenon is of more general significance. Steitz and Jakes describe an experiment in which ribosomes were allowed to bind to intact R17 before exposure to RNase: the three initiation regions were then isolated as ribosome-protected fragments Although the A protein initiation site shows the greatest complementarity with 16S rRNA, it is the site least favoured by this ribosome binding /protection reaction. Other, stronger signals direct ribosomes preferentially to the replicase and coat protein initiation sites. There is evidence from ribosome reconstitution experiments suggesting that, in addition to 16S rRNA, the ribosomal protein S12 plays a role in recognising the other initiation sites (Held *et al*, *Biochemistry*, **13**, 2115–2122; 1974) Whatever signals are recognised in the replicase and coat protein initiation regions, they appear to lie outside the region conventionally isolated as a ribosome-protected fragment because, of the isolated fragments, only the A protein initiation fragment can bind efficiently to ribosomes (Steitz, *Proc. natn Acad. Sci. U.S.A.* **70**, 2605–2609; 1973).

This is not to say that other recognition signals do not lie within the ribosome-protected regions, only that in the case of the R17 synthetase and coat protein genes, these are not sufficient by themselves to permit efficient ribosome binding. Other features shared by many ribosome-protected fragments have been noted by Pieczenik *et al.* (*J molec. Biol.* **90,** 191–214; 1974) in their studies on ribosome-binding sites from phage f1. First, the sequence coding for the first few amino acids often contains termination triplets in the two unused reading frames. These might serve to abort peptide bond formation after an out of phase initiation. Any feature characteristic of an initiation site, however, might be utilised in the recognition process and indeed, a role for the 3′ end of 16S rRNA in recognising termination triplets was postulated by Shine and Dalgarno (*Proc. natn. Acad. Sci. U.S A.,* **71,** 1342–1346; 1974). The other feature of coding sequences within initiation sites is palindromic sequences, that is, sequences such as C-A-G-G-A-C which 'read' the same in both directions. It is difficult to view palindromes as recognition elements since it would seem that they can only be identified by those who can read. In the absence of any evidence, both the out of phase termination triplet and the palindromic sequences should be considered characteristics of initiation regions. The only features presently identifiable as recognition signals are the 'caps' in eukaryotic mRNAs and the sequences complementary to 16S rRNA in prokaryotic mRNAs

If the Shine–Dalgarno base-pairing signal is an important feature of initiation sites, strong selective pressures would act against any change in the relevant rRNA sequence This sequence, however, has diverged somewhat within bacterial species and, in eukaryotes, the 3′ end of 18S rRNA bears no resemblance to that of 16S rRNA, although this sequence is apparently invariant within the eukaryotes Whether or not a similar scheme exists in eukaryotes is debatable. Dasgupta *et al.* (*Nature,* **256,** 624–628; 1975) have determined the nucleotide sequence of the initiation region of brome mosaic virus RNA-4—the mRNA for BMV coat protein. The sequence exhibits a weak, four-base complementarity with the 3′ end of 18S rRNA. More interesting is the manner in which this region was isolated. The BMV RNA was subjected to limited RNase digestion and the fragments so produced were bound to wheat germ ribosomes. The region used for sequence analysis was then isolated from the 80S ribosomes as the only bound fragment which was annealed to the rRNA—the coding properties of this fragment demonstrate that it is indeed the authentic initiation region of this mRNA. A similar observation suggesting a functional role for mRNA: rRNA base-pairing comes from Kabat (*J. biol. Chem ,* **250,** 6085–6092; 1975). Kabat isolates rabbit globin mRNA complexed to rRNA and finds that this complex is more active in translation by RNase-treated ribosomes than is the free mRNA. It is tempting to speculate that the RNase treatment has removed an exposed region of the rRNA which functions in initiation site recognition and that the exogenous rRNA can substitute for this missing sequence. It should be noted, however, that both 18S and 28S rRNA show this potentiation activity. Further studies with eukaryotic mRNA species will be required before conclusions can be drawn with any confidence.

What then is an initiation site? In bacterial systems it can be a fairly extensive region possibly extending into coding sequences. Within this region there may exist a number of features of primary or secondary structure which serve to direct ribosome binding. These signals must be available to ribosomes and not masked either by mRNA secondary structure or by proteins bound to the message. This latter may be an important control point regulating the synthesis of individual proteins in situations where transcriptional control is not appropriate (as in the RNA phages or in early embryogenesis). It is likely that as more extensive sequence information becomes available we will come to recognise more of the features responsible for directing the binding of regulatory proteins and ribosomes. ☐

Transform faults to spreading offsets

from Peter J. Smith

The term 'transform fault' has become an integral part of the language of the Earth sciences. It was introduced by Wilson (*Nature,* **207,** 343; 1965) to describe a fault that terminates abruptly at points where its motion is 'transformed' into a movement of a quite different feature, and was first used specifically in connection with offset spreading oceanic ridges, where strike–slip motion is limited to the zone between the two ridge segments. But this is a purely phenomenological definition carrying no information about how such faults originate. And therein lies a problem which according to Vroman (*Tectonophysics,* **30,** T11; 1976) has led to a "mire of misunderstandings".

The difficulty is that the, albeit necessary, adoption of a descriptive definition has apparently encouraged many workers to assume that all faults conforming with it have the same cause. Not that the cause has been established, even for the familiar transform faults in the ocean floor. Shear fault patterns on land are the result of brittle fracturing in an elastic solid, and it has therefore seemed reasonable to many to assume that the dense grid of 'shears' representing transform faults in the solid ocean floor is produced likewise In Vroman's view, however, this is not necessarily the case. On the contrary, the lack of a conjugate set in the pattern of oceanic transforms suggests that these offsets were created in material having Newtonian properties and responding to the laws of laminar flow. The apparent paradox that transform faults are now found in solid crust is then to be resolved by assuming that the faults are inherited from a time before the Newtonian liquid's solidification.

To develop these ideas, Vroman refers back to the original laboratory experiments of Oldenburg and Brune (*Science,* **178,** 301; 1972) who showed that spreading axes, transform faults and their associated fracture zones could be generated in freezing paraffin wax. One of the important results obtained by Oldenburg and Brune was that whereas the liquid material upwelling along a regular spreading axis solidifies and enlarges each plate equally, accretion takes place differentially along an initially jagged axis in such a way as to smooth out the irregularities According to Vroman's interpretation of the Oldenburg–Brune model, however, spreading axes also exhibit more prominent irregularities which are essentially everlasting.

These 'kinks' are thought to play an important part in the development of the spreading pattern. As liquid is carried away to the two accreting walls of the spreading axis, a flow pattern is induced. But the kinks in the walls exert a frictional force on the liquid and thus produce finite differences in flow velocity which perturb the accretion process. The lines connecting corresponding kinks become zones of weakness which may fracture with the slightest encouragement, such as might be given by small thermoelastic stresses during the final stages in the cooling of the liquid. In short, Vroman suggests that there is a 'rate of shear' between neighbouring stream tubes which produces transform faults parallel to the stream lines at the liquid surface. On the other hand, some transform faults are evidently not related to kinks. In such cases, shear may result not from irregularities in the spreading axis walls but more directly from irregularities (inhomogeneous viscosity, for example) in the intervening liquid itself.

Vroman as good as admits that his interpretation of the Oldenburg–Brune model may be contentious, but as further evidence, points to the Gulf of Aden where transform faults can be seen to connect corresponding kinks in the opposing shelf margins. Moreover, this real example adds a further dimsension to the argument in that the kinks here are old and evidently precede the onset of spreading; they presumably occur where the new spreading axis intersects an old fault system. To this extent, therefore, transform fault locations may be governed by the prespreading history of the area concerned.

But be that as it may, Vroman's analysis clearly applies to the transform faults of the ocean floor. At the same time, however, there are several transcurrent faults in solid continental rocks which, because they happen to terminate at structural features which 'absorb' their motion, must also be termed 'transform faults' according to Wilson's strict definition. In other words, Wilson's definition includes faults of two quite different origins; and it is this ambiguity which has led to the misunderstandings and even "evil consequences".

It would appear to Vroman therefore that the term 'transform fault' is no longer restrictive enough to be useful. The unique character of oceanic 'transform faults' lies not in their transforming properties but in their origin. For this reason, Vroman proposes that the term be abandoned in the oceanic context and replaced instead by the name 'spreading offset'. □

Progress with X rays

from Andrew Fabian and James Pringle

On January 27–29 about 200 astronomers and physicists assembled at the Massachusetts Institute of Technology for a meeting of the High Energy Astrophysics Division of the American Astronomical Society. The subect was X-Ray Astronomy.

THE morning sessions were taken up with invited contributions, several of which were genuine and useful reviews of the invited topics. Too many speakers, however, provided an unbalanced view of the field with particular emphasis on their own achievements. The content of the contributed papers reflected the attitude of the conference organisers to theoretical matters. Of more than 70 short contributions accepted by the organisers, only three were on theoretical topics. This meant that not only was theory underrepresented (and possibly misrepresented), but also that a number of the supposedly observational contributions contained more than their advertised share of theoretical explanation, some of it of dubious quality. Most of the contributed papers were of high quality and several interesting new results were presented.

The body of data on the pulsing X-ray sources is increasing. Most of the periods are in the range of minutes, but R. Lucke (Naval Research Laboratory, Washington DC) reported that the X rays from the source in the small Magellanic cloud, SMC X-1, are pulsed with a period of 0.716 s. The pulsed fraction is about 25% between 1.6 and 7 keV, and possibly variable at lower energies, when data from a rocket flight and the Apollo spacecraft 1.5 yr later are compared. S. Rappaport (MIT) showed preliminary results on the changing period of the pulsed transient source A0535+26, although the observations are not complete or sufficiently extensive to be very useful. G. Fabbiano (Center for Astrophysics, Harvard University) reported the discovery of a glitch, giant by pulsar standards, in the pulse period of Cen X-3. The period changed by about two parts in 10^5 in less than a day. W. Forman (Center for Astrophysics) has analysed some of the later Uhuru data and 23 new weak galactic sources have been found. The 'extended low' in the 35-d cycle of Hercules X-1 contains a phase where pulsed emission is detected at about 30% of the level of the normal 'on-state'. C. Jones (Center for Astrophysics) stated that this feature has been detected every time that this phase has been observed.

Evidence is also growing that sporadic burst behaviour may be a common feature in the X-ray sky. R. D. Belian (Los Alamos Scientific Laboratory) reported that a number of bright X-ray flares of a few minutes duration had been seen by the two Vela 5 satellites, possibly all coming from a region in the constellation of Norma. Several speakers reported transient X-ray behaviour at high galactic latitudes, and G. Jernigan (MIT) presented data on the 10-s flares first seen by the ANS satellite (Grindlay *et al.*, *Int. astron. Union Circular* No. 2879, 1976), from the direction of the globular cluster NGC 6624. A sequence of nine flares has been seen, recurring with an average of 15,718 s, although there appears to be a jitter of up to 1,000 s. Several other flares have been observed near this region of the sky, but directional information excludes the globular cluster.

One striking feature of the meeting was the quality of the spectra obtained from the Ariel 5 satellite. L. Culhane (Mullard Space Science Laboratory, University College London) showed that the spectral data now acquired from clusters of galaxies require more sophisticated models than the simple one-temperature thermal bremsstrahlung fits previously used. Indications of an emission line, possibly due to iron, in the spectrum of the Perseus cluster provided a foretaste of possible abundance analyses in such distant objects. P. J. N. Davison (Mullard Space Science Laboratory) and S. H. Pravdo (Goddard Space Flight Center, Greenbelt) showed clear evidence for iron line emission from the Cassiopeia A supernova remnant, as viewed by the Ariel 5 and OSO-8 satellites, respectively. Another glimpse of the future was provided by P. Gorenstein (Center for Astrophysics). He presented an X-ray picture of the Perseus cluster obtained with an imaging X-ray telescope from a rocket flight lasting a few minutes. The launch of a similar instrument on the satellite HEAO-B must revolutionise the present hazy knowledge of the X-ray sky. □

The brain of the "reeler" mouse

from Shin-Ho Chung

Recent anatomical studies on neurological mutant mice have provided fascinating insights into the sequence of events leading to the formation of the brain. Before making synaptic contacts with one another, young neurones migrate from an inner mitotic layer to their eventual destinations where they form laminar aggregates. Because a defective gene in the mutant mouse disrupts a certain stage of this complex and precisely ordered sequence of events, analysis of the affected brain gives insight into both the action of the mutant gene and the normal mechanisms controlling neurogenesis. In some mutants, the genetic disturbance is confined to a specific region of the brain (News and Views, *Nature*, **257**, 86; 1975), whereas in others, such as the "reeler", the consequences of the defective gene can be noted in a variety of different cortical structures.

The autosomal recessive "reeler" gene arose by spontaneous mutation, and the homozygous mutants have an unsteady gait suggestive of inebriation (Falconer, *J. Genet.*, **50**, 192; 1951). Histological examination has revealed that cellular organisation in the brain is grossly disrupted, especially in the cerebellum, the hippocampus and the neocortex (Meier and Hoag, *J. Neuropathol. exp. Neurol.*, **21**, 649; 1962; Hamburgh, *Dev. Biol.*, **8**, 165; 1963;

Cragg, *Expl. Neurol.*, **49**, 858; 1975). Instead of forming tightly packed layers oriented uniformly towards the cortical surface, cells in the corresponding layers of the "reeler" are very loosely packed and randomly oriented. Caviness and Sidman (*J. comp. Neurol.*, **145**, 85; 1972; *ibid*, **147**, 235; 1973) have noted in the "reeler" a second systematic error in that the loosely packed layers are inverted relative to their positions in the normal brain. In the olfactory cortex, for example, the larger pyramidal cells are below an outer zone of small polymorphic cells, which is the reverse of the order in the normal brain.

The failure to achieve the normal laminar aggregates in the mutant brain is not caused by an abnormality in the time of cell generation or the ability of cells to migrate in their normal sequence (Caviness, *J. comp. Neurol.*, **151**, 113; 1973; Devor *et al.*, *ibid*, **164**, 471; 1975) Autoradiography has shown that each class of cells in the "reeler" undergoes final mitosis at the normal time during embryonic development, and the onset of migration into the cortical plate also proceeds normally. During the terminal phase of migration, however, the mutant gene misdirects morphogenetic events governing cell position. Normally, newly mitosed cells migrate outwards towards their final position, with successive layers being formed by younger cells migrating through previously laid-down strata of older cells. Thus, in the normal animal, the younger the cell the further it has to migrate to its final destination. In the "reeler", this process is reversed. Instead of stopping at the correct location, the first wave of migrating cells advances to the superficial layers, and subsequently generated cells take up deeper positions.

In spite of this eversion of cell layers resulting from aberrant cell migration, subsequent developmental processes appear to proceed normally. Trajectories of the principal axons to their appropriate regions are sometimes altered to seek out remote abnormally positioned cells (Frost and Caviness, *Proc. A. Meeting Soc. Neurosci.*, **4**, 217; 1974). In other regions where the path of growing axons remains unaltered, displaced cells compensate for the misalignment by extending their dendrites further than they normally do, meandering tortuously until the correct input is found (Devor *et al.*, *op. cit.*; Bliss *et al.*, *Brain Res.*, in the press). When examined electrophysiologically, the patterns of cell to cell connectivity are remarkably like the normal, at least in the hippocampus (Bliss and Chung, *Nature*, **252**, 153; 1974). Thus the brain appears sometimes to make use of compensatory mechanisms to establish functional integrity.

That a single gene mutation causes the same sort of cellular disarray in very different parts of the brain suggests that there is a developmental mechanism with a unique molecular basis that is common to them all. It has been widely conjectured that the interaction between migrating young neurones and their input axons from other parts of the brain is crucial for the normal ordering of neuronal networks. In many areas, these input axons reach their appropriate sites coincidentally with or in anticipation of the arrival of their target cells. Such a precisely synchronised afferent–efferent interaction may be at fault in the "reeler". For example, the axons may be delayed in arriving or the young cells themselves may fail to interact properly with their partners. Alternatively, migrating cells may lack a surface property which facilitates aggregation once they have reached their destination. This line of speculation leads to a biochemical search for a missing or altered protein on the cell surface. In this context, it is relevant that cortical cells cultured *in vitro* from "reeler" mice do not aggregate to form the laminar arrays characteristic of cells from normal littermates (DeLong and Sidman, *Dev. Biol.*, **22**, 584; 1970). The sequence of molecular events linking the "reeler" gene to the abnormal pattern of cell migration will undoubtedly be unravelled; and the elucidation of such a process will be a landmark in the understanding of how the brain is formed. □

Deformation within continental plates

from J. Sutton

Now that it is possible to measure the very small movements that occur within continental plates a variety of researches become feasible. As a group of papers read at the fifth international symposium on Recent Crustal Movements (*Tectonophysics*, **29**; 1975) shows, the relations between movement in the Rhineland and the regional stress field produced in Central Europe as a result of the movements in southern Europe and the opening of the Atlantic are now well established. Compressive stress acting on north-west and south-east lines produces slip at rates of 0.05–0.3 mm yr^{-1} in the Rhineland valley. These movements, slow by comparison with those in California or New Zealand, which may be 500–1,000 times as great, are accompanied by shallow earthquakes which occasionally cause damage and deaths as at Basel in 1356 when 300 persons were killed. Ahorner (*Tectonophysics*, **29**, 233; 1975) emphasised how small are the movements which explain the seismicity of the region. At greater depths, flow of rock is important and Illies (*Tectonophysics*, **29**, 251; 1975) described how the mantle has risen for 6 km into the crust below the Rhinegraben rift system. Illies suggested that subcrustal masses displaced by plate collision, induced volcanism and intraplate rifting north of the Alps.

In last week's issue of *Nature* (page 648) Coward discussed another aspect of intraplate tectonics, the possible influence of pre-existing structures on present day movements. He described structures from near Adelaide, an area like southern Germany in that it lies between a plate boundary, the Pacific margin, and an ancient stable area in West Australia. In Australia as in Europe, recent volcanics are virtually absent where very old rocks outcrop (western Australia, Sweden and Finland), but are sporadically distributed through parts of both continents which have been deformed since the Precambrian.

Coward described deformation within a well known belt of folded rocks east and north of Adelaide. He has determined the extent of deformation across a belt some 150 km wide where shearing about 500 Myr ago produced displacements which he estimates to amount to 150 km. Making comparisons with other belts of intensely deformed rock formed by shearing parallel to their length, Coward suggested that the South Australian belt might have extended at least 10 times the length of such displacement. Such a long structure might be expected to affect both upper mantle and crust.

Coward went on to make the point that the Adelaide region is a zone of recent seismic activity and referred to the work of Cleary and Simpson (*Nature*, **230**, 239; 1971), in which they considered the distribution of earthquakes within the Australian continent, and where they discussed three seismic zones within Australia that could be lined up with sections of the Antarctic Ridge where earthquakes occur along offsets. One of these zones which passes near Adelaide and which follows the Ordovician structures mentioned by Coward, continues, Cleary and Simpson suggested, into the most westerly of the offsets in the oceanic ridge recognised by Sykes (*J. Geophys. Res.*, **75**, 5041; 1970) off South-East Australia. The implication is that the present day seismicity follows lines of weakness established within rock deformed much earlier in the geological history of Australia.

Such a relationship may illustrate a

conclusion drawn by Watterson (*Nature*, **235**, 521; 1975), that large scale ductile simple shear belts in which deformation is usually accompanied by a reduction in grain size, may produce a permanent weakness in the crust. Watterson suggested, following work in West Greenland, that diffusion at grain boundaries plays an important role in ductile deformation, and the reduction in grain size with the consequent increase in such boundaries permanently reduces the strength of rock so deformed. The South Australia belt discussed by Coward might have been weakened in this way (also see Dunnet, *Phil. Trans. R. Soc., Lond.*, **A280**, 641; 1976). Coward suggested that the transform fault which offsets the Antarctic ridge south of Adelaide formed where the zone of ductile deformation was crossed in early Tertiary times by the spreading axis from which Australia and Antarctica were to separate. Cleary and Simpson suggested that the pattern of seismicity within Australia—essentially a belt in the south-west, another passing through Adelaide to the centre of the continent and on to the western coast and a third and more diffuse zone running parallel to the Pacific coast from Victoria to southern Queensland—could be accounted for by contrasts between the ancient shield to the west and a relatively "soft" layer beneath south-eastern Australia. They noted evidence suggesting a strongly developed low velocity layer and probably, therefore, a substantial zone of low strength in the upper mantle below south-eastern Australia. The observations of Coward and Watterson indicate that belts of ductile shear which probably extended down to the base of the crust could introduce a further weakness into the lithosphere which would be reflected in the distribution of earthquakes, and which might have influenced the many Tertiary volcanic centres of eastern Australia. □

Spectrin-like proteins

from Dennis Bray

A RESEARCH worker at the Pacific Research Center, Honolulu, tries to prepare tubulin from sea urchin eggs by a standard method and obtains an unexpected result. The extract obtained with glycerol and a chelating agent forms a gel on warming as usual, but this is made of filamentous actin instead of microtubules. The actin is combined with two other proteins, one of which has a molecular weight of 220,000 (Kane, *J. Cell Biol.*, **66**, 305; 1975). A second investigator, by a different ocean, isolates the acrosome of an echinoderm sperm. This is composed of unpolymerised actin and a high molecular weight protein with a similar behaviour on SDS–acrylamide to spectrin—the major structural component of mammalian erythrocyte membranes. The author suggests that the two might be related and, in collaboration, shows that human spectrin and rabbit muscle actin can interact (Tilney, *J. Cell Biol.*, **63**, 349a; 1974; Tilney and Detmers, *J. Cell Biol.*, **66**, 508; 1975).

Within a year two other laboratories find that they too can obtain clear watery extracts which set when warmed. One is from *Acanthamoeba*, the other from rabbit lung macrophages. In both of these the gelled material comprises actin together with one or two other proteins. The purified high molecular weight protein from macrophages binds directly to F-actin and is compared with spectrin (Pollard, *Biophys. J.*, **15**, 124a; 1975; Hartwig and Stossel, *J. biol. Chem.*, **250**, 5659; 1975). A new high molecular weight protein is isolated from smooth muscle. It is a major component and present in kidney, lung, liver and brain, but not skeletal muscle. On immunological evidence it is associated with the fibrillar arrays within cultured cells. It is not myosin and the authors compare it to spectrin and the recently described actin-binding protein of rabbit macrophages (Wang *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4483; 1975).

The casual reader of these papers, trying to keep up with the breathless pace of developments in cell motility, will find them intriguing in content and captivating in style. He will also, unless he is careful, come away with the wrong conclusion. For, although each is distinct and in essential details quite different, there is a strong underlying similarity in approach and interpretation (which has been emphasised in the gloss above). Although it is not explicitly said, there are broad hints from the *dramatis personnae* that there is a moral. The audience is led to make its own conclusions, one version of which is that a high molecular weight protein, similar to spectrin, is present in many kinds of cell and influences the polymerisation of actin. But is this justified? Let us examine the evidence.

To begin with the new proteins are not necessarily the same. They are grouped together under the generic description of 'high molecular weight' but in a way this is misleading. It implies that there is some structure or function that always goes with a polypeptide of more than 200,000 daltons, and we do not know this to be true. There is no reason why the molecular weights of these proteins should not be as precisely compared as any other—but so far this has not been done. At present they could vary by as much as 30,000 daltons: which as evidence of identity is not compelling. Other properties, such as solubility and actin-binding are not quantitative in the present context and, moreover, show differences as well as similarities. Second, these proteins are not spectrin. Of those that have been compared directly on SDS–acrylamide gels, two are clearly heavier and one overlaps in only one of its components. The other obvious test—that of immunological cross-reaction—has so far not been reported, although at least one of the laboratories involved must have all the necessary ingredients. In fact, less than a year ago they used an antibody to spectrin to demonstrate that this protein is absent from most tissues (Painter *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1359; 1975). As to whether the proteins are 'spectrin-like'—the question is as insubstantial as it sounds. Criteria which have been advanced in support of this aetherial quality are high molecular weight (see above), solubility in low ionic strength solutions and 80% ethanol, absence of ATPase activity and failure to form filaments in isotonic buffers. Finally, they are not necessarily involved in actin polymerisation, although it would be of great interest if they were. The possibility arises because all but one of the proteins is extracted together with actin which later polymerises, but the direct evidence, which can only come from tests with purified components, is so far lacking. Therefore, although these new and noteworthy phenomena are clearly related, it is too early to say in what way. In particular, a simple explanation of the kind given above is at present untenable. □

A hundred years ago

THE *Morgenblad* of Christiania states that a singular phenomenon was observed there after a recent violent storm A number of worms were found crawling on the snow, and it was impossible to find the places from which they had issued, everything being frozen in the vicinity. Similar circumstances were reported from several places of Norway.

MANY of our readers, we are sure, will rejoice to hear that a movement is on foot in Germany to abolish the crabbed printed German alphabet and adopt Roman type. We sincerely wish the movement may lead to the desired result, and that it will extend to the still more vexatious written alphabet. from *Nature*, **13** (March 2), 357; 1876.

articles

Suppression of malignancy in human cells

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Fusion between malignant and normal human cells results in the suppression of malignancy as measured by tumour formation. Malignant–malignant hybrids retain their malignant character. The human cell system has advantages for the study of the control of malignancy.

BOVERI proposed that malignant expression may originate from chromosome imbalance[1] and there is little doubt that the transition from a normal to a malignant cell involves alteration of cellular regulation events, which presumably stem from changes in the genome. Such alterations could conceivably be induced by carcinogens or oncogenic viruses, or arise spontaneously as a result of somatic mutation. It is possible, therefore, that a normal cell becomes malignant on addition of genetic material, as with oncogenic viruses, or because a genetic regulating function becomes inactivated, or because of a combination of these events[2].

Genetic analysis of malignancy (defined here as the ability of cells to proliferate and produce tumours in appropriate hosts) has been facilitated by the ability to hybridise somatic cells from the same or different animal species. The work of Barski, Ephrussi and associates[3–5], using hybrids between highly malignant and normal or low malignant mouse cells, suggested that malignancy was a dominant trait; that is, all hybrid cell populations produced tumour in suitable hosts. Later, however, Harris, Klein and associates[6–12] demonstrated convincingly that malignacy is suppressed in hybrids between malignant and non-malignant or low malignant cells and that malignancy behaves as a recessive trait in these conditions. This disparity in results was later recognised as being due to the loss of chromosomes from the respective hybrid populations[6–13]. Based on extensive chromosome analysis, Harris *et al.*[7–12] proposed that a specific chromosome(s) is involved in the suppression of malignancy, and that when this chromosome(s) is lost the malignant trait is re-expressed. The frequency of production of tumours by hybrids between malignant and normal cells was apparently related to the stability of the chromosomes of the hybrid population.

Most reported genetic analyses of malignancy have involved intraspecific cell fusion, using cells of rodents and other species. I now wish to report the suppression of malignancy in human–human cell hybrids and discuss some of the advantages of this system.

Parent and hybrid cells

The malignant cell populations used were two HeLa variants D98/AH-2 and HBU which lack detectable hypoxanthine guanine phosphoribosyl transferase and thymidine kinase activity, respectively[14,15]. Actively proliferating or senscent diploid human fibroblasts were used as the normal parent populations. An inoculum of 1×10^5 cells of either HeLa parent produces tumours in 100% of inoculated animals, whereas 5×10^7 normal fibroblasts produce no tumours (unpublished).

Fusion of cells was accomplished with inactivated Sendai virus according to Harris and Watkins[16]. The procedures used to select hybrids are outlined in Table 1. Briefly, malignant–malignant hybrids between the two HeLa parents were selected in HAT medium[17]. As the normal diploid population had no genetic markers we used three semiselective techniques. (1) HeLa variants were fused with senescent fibroblasts which had lost their proliferative capacity and hybrids were selected in HAT medium. HeLa parent cells died in HAT and the senescent fibroblasts would not grow in any growth medium; thus only HeLa–fibroblast hybrids survived and replicated. (2) Since it could be argued that normal cells at the end of their proliferative lifespan have accumulated errors that interfere with their normal regulatory functions[18], actively dividing fibroblasts were treated with iodoacetate. Cells treated with iodoacetate die unless rescued by fusion with untreated whole cells (in this case HeLa variants)[19]. Hybrids were again selected in HAT medium. (3) HeLa–normal fibroblast mixtures at a ratio of 10,000 : 1 were fused and hybrids were selected in HAT medium. This semi-selective procedure took advantage of the fact that human fibroblasts divide very slowly at low population densities. Rapidly dividing hybrid clones were detected readily and removed from the few contaminating fibroblasts.

The morphological characteristics of the mass populations of parent and hybrid cells are illustrated in Fig. 1. The malignant–malignant hybrid ESH6 (Fig. 1*d*) retained the epithelial-like morphology of the two parental HeLa variants (Fig. 1*b* and *c*). The only distinguishing features were increased cell size and larger nucleoli. All the malignant–normal hybrids had a morphology intermediate between that of the epithelial-like malignant parent and the fibroblastic normal parent (ESH5 only illustrated in Fig. 1*e*). This intermediate morphology has been retained in mass culture for several months and at least 60 population doublings. Clones were selected, however, which more closely resembled either the fibroblastic or epithelial-like parent (Fig. 1*f*).

In their organisational behaviour the malignant–normal

Table 1 Selection of human cell hybrids

Hybrid designation	Parent cells	Selective medium
ESH2	D98/AH-2 + senescent fibroblast	HAT*
ESH3	D98/AH-2 – iodoacetate-treated fibroblast	HAT
ESH5	D98/AH-2 + proliferating fibroblast 10,000:1 ratio	HAT
ESH6	D98/AH-2 + HBU	HAT

* HAT medium is Eagle's basal medium supplemented with 10% foetal calf serum, 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine.

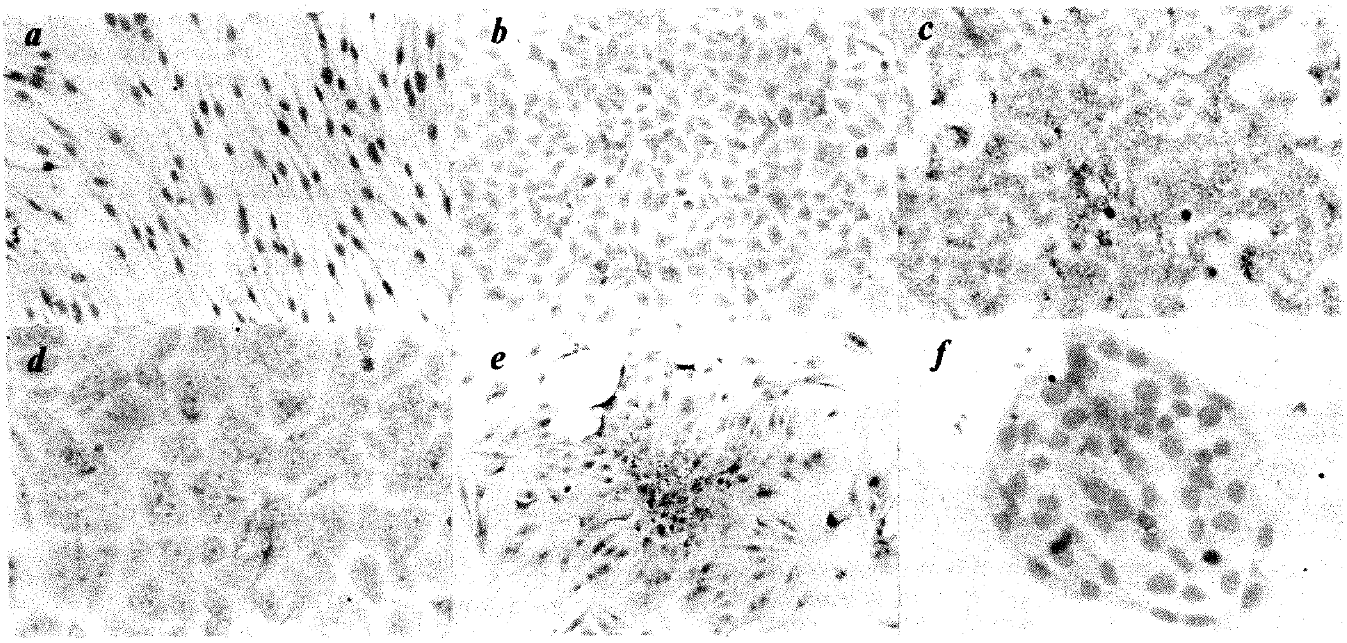


Fig. 1 Morphology of parent and hybrid cell populations. *a*, Normal fibroblast; *b*, malignant parent D98/AH-2; *c*, malignant parent HBU; *d*, malignant–malignant parent ESH6. The hybrid retains the epithelial-like morphology of parent cells shown in *b* and *c*. *e*, Malignant–normal hybrid ESH5. Morphology is intermediate between that of the parent cells shown in *a* and *b*. *f*, Clone of malignant–normal hybrid ESH3. Cells more closely resemble the malignant parent D98/AH-2.

hybrids more closely approximated that of the malignant parent cells. There seemed to be no density-dependent inhibition of division and the cells grew to high population densities (Fig. 2). In mass culture or in clones, however, the cells generally tended to be loosely packed and aligned themselves on their long parallel axes. Studies to be reported elsewhere have shown that the sensitivity of these hybrids to serum growth factors is intermediate between that of the malignant and normal parent cells. The malignant–malignant hybrids behaved in all respects like their malignant parent cells.

Chromosome constitution and assay for malignancy

Chromosome analyses of parental and hybrid cells are presented in Table 2. All the normal fibroblast cell strains used had a stable diploid karyotype. D98/AH-2 and HBU both had a relatively narrow modal distribution with means of 61 and 57, respectively (Fig. 3). The earliest time when various hybrids could be analysed was 4 weeks after fusion. In all cases, the mean chromosome constitution was slightly less than the expected mean calculated for the retention of the total complement of chromosomes from both parents. Thus, as in other animal cell systems[20], there seemed to be early loss of chromosomes from hybrids. Whether the loss was random or non-random with respect to specific chromosomes has not been determined. After the early segregation events the chromosome content of the hybrids stabilised. We have observed very little loss of chromosomes in 5 months, during which the hybrid populations underwent at least 70 doublings (see, for example, ESH6 data in Table 2 and Fig. 4).

A problem when human cells are used for analysing malignancy is to find a suitable host in which to test the malignant potential of such cells. We have reported that human and animal malignant cell lines and biopsied human tumour material established and produced tumours in immunosuppressed mice, whereas normal cells failed to proliferate[21,22]. We also found that when mixtures of malignant and normal cells were inoculated, only malignant cells proliferated to form a tumour whereas the normal cells failed to survive. We have purified malignant cell populations from mixtures of normal and malignant cells by this procedure[22]. We used three types of immunosuppressed

Table 2 Chromosome analysis of human parent cells and hybrid derivatives

Cell type	Range Expected*	Range Observed	Mean Expected*	Mean Observed
Parent				
Normal fibroblasts	46	45–47	46	46
HeLa D98/AH-2	—	50–64	—	61
HeLa HBU	—	47–66	—	57
Malignant–normal hybrids				
ESH2 6 weeks after fusion	96–110	90–107	107	101
ESH3 4 weeks after fusion	96–110	94–106	107	104
ESH5 4 weeks after fusion	96–110	95–105	107	102
Malignant–malignant hybrid ESH6				
4 weeks after fusion	97–130	95–124	118	110
20 weeks after fusion	97–130	90–114	118	106
ESH6T 20 weeks after fusion†	97–130	95–118	118	107

Counts were based on at least 30 metaphase spreads.

* Estimated range and mean assume the presence of a full complement of chromosomes from the respective parent cells.

† Includes a total of 13 weeks in the mouse followed by re-establishment of tumour cells *in vitro*.

mice in the study reported here, all of which gave comparable results. The mice were (1) thymectomised neonatally and treated with antithymocyte serum; (2) weanlings that had been thymectomised, whole-body irradiated and reconstituted within 2 d with bone marrow or foetal liver cells[23]; and (3) nude mice that require no immunosuppressive treatment because they lack a functional thymus and cannot mount a significant cell-mediated response against foreign antigens[24].

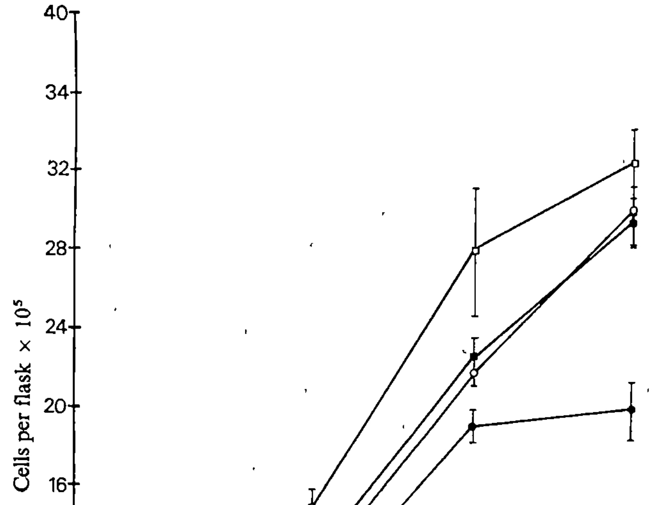
The results of the malignancy assays are presented in Table 3. Clearly only the malignant–malignant hybrid ESH6 retained its malignant properties. The fact that comparable cell numbers of ESH6 and the parent HeLa variants formed tumours is an indication that there was no partial loss of malignancy in the ESH6 hybrid. This was further substantiated by comparing the karyotypes of cells re-established *in vitro* from ESH6 tumours and ESH6 cells grown only *in vitro* for the same time (Fig. 4). The karyological profiles in Fig 3*b* and *c* are very similar, indicating that there was no selection *in vivo* for a malignant subpopulation of the total ESH6 mass culture.

The suppression of malignancy in the malignant–normal cell hybrids seemed to be complete; as many as 1×10^7 cells inoculated failed to produce a tumour. Periodic monitoring of these cell populations during 6 months has yet to reveal a single tumour. It should be emphasised that only mass cultures have been tested. We are examining the malignant traits of a series of clones of different morphologies derived from the malignant–normal hybrid populations.

Malignancy as a recessive trait

The studies reported here clearly demonstrate that malignancy behaves as a recessive trait in human cells, and support the findings of Harris *et al.* who demonstrated suppression of malignancy in mouse cell hybrids. Because of the preliminary nature of this report, it is not possible to conclude whether suppression is related to a specific genetic defect in the malignant cell which is made good by complementation with a normal cell[8] or to a balance of chromosomes carrying information for expression or suppression of malignancy as proposed by Sachs and others[25–27].

Fig. 2 Growth curves of parent and hybrid cell populations. 2.5×10^5 cells were inoculated into T-25 flasks (Falcon). Triplicate samples were taken at each time and counted in a haemocytometer. ●, WI-38; □, D98/AH-2; ○, ESH5; ■, ESH3.



Weiner *et al.*[10,12] found that several different malignant mouse cell lines failed to complement when fused together. This startling finding seems to suggest that malignancy in different cancer cells is due to a defect at the same genetic locus. My studies reported here do not help to clarify this intriguing possibility because the two HeLa variants used for the malignant–malignant fusion presumably originated from the same cancerous clone.

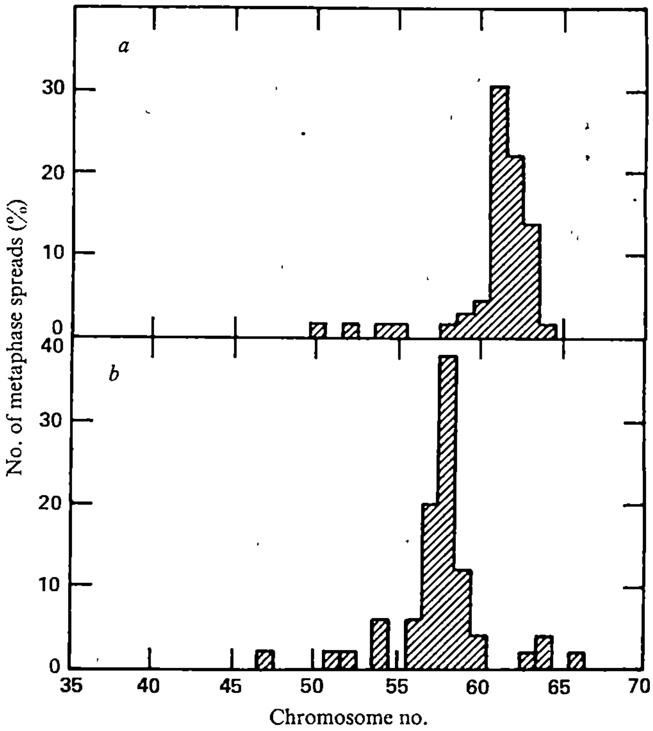


Fig. 3 Modal chromosome number of D98/AH-2 and HBU. *a*, D98/AH-2; *b*, HBU. At least 50 metaphase spreads were counted for each determination.

My results are at variance with those of Croce *et al.*[28] who reported that hybrids between SV40-transformed human cells and mouse macrophages retain the expression of SV40 tumour (T) antigen and are tumorigenic as long as the human C7 chromosome is retained in the hybrid. Loss of C7 was correlated with loss of T antigen and loss of tumorigenicity. Their findings indicate that the SV40-transformed phenotype is under positive control[29]. The fundamental difference in our results may be explained on the basis that Croce *et al.* used human–mouse interspecific hybrids which may involve different regulatory factors. Also, the malignant human cells were transformed by an oncogenic DNA virus, SV40. The viral genetic information necessary for this transformation event may be able to supersede the regulatory control exerted by normal gene(s) which are necessary for the suppression of malignancy.

My studies with malignant–normal hybrids indicate that senescent normal fibroblasts can be rescued by fusion with malignant HeLa cells and attain immortality. Thus senescence of human fibroblasts in culture[30,31] behaves as a recessive trait when fused with HeLa cells, a phenomenon

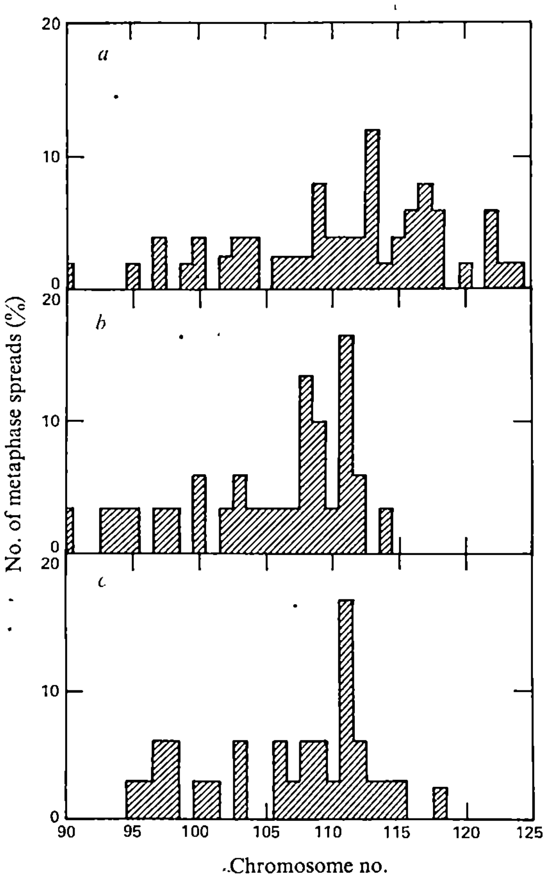


Fig. 4 Modal chromosome number of ESH6 at various times after selection. *a*, 4 weeks after fusion; *b*, 20 weeks after fusion; *c*, 20 weeks after fusion, including 13 weeks in a mouse. At least 50 metaphase spreads were counted for each determination

noted by others[32,33]. But although the malignant–normal hybrids can proliferate indefinitely, they are not malignant. The use of these human–human hybrids may provide a method whereby transformation events can be separated from the event(s) leading to malignancy.

The human–human hybrid cell system has several advantages over the murine or interspecific systems for the analysis of malignancy. (1) Most normal diploid mouse cell strains spontaneously transform into heteroploid malignant cell lines, thus normality is not a stable characteristic of mouse cells grown *in vitro*. Normal human fibroblasts have a stable diploid karyotype and a limited *in vitro* lifespan[31], and have not been shown to transform spontaneously. (2) Many hybrids between malignant and normal mouse cells retain their malignancy when injected into animals[7]. This apparent lack of suppression of malignancy has been shown to be due to rapid chromosome segregation from the hybrid cells. The rapidity of this chromosome loss, in addition to making the initial premise of suppression of malignancy hard to evaluate, renders the identification of specific chromosome(s) controlling this expression extremely arduous. My findings indicate that human cell hybrids have very stable chromosome constitutions which should render the isolation and characterisation of malignant variants easier. (3) Most, if not all, mouse cells contain endogenous RNA tumour virus genomes[35,36], but claims for the presence of human RNA tumour viruses in human cell lines[37,38] have been unsubstantiated and probably represent contamination by viruses from other animal hosts[39]. (4) Certain claims for the suppression of malignancy in mouse cell hybrids have been shown to be due to histoincompatibiity rather than a genetic regulatory element[40] Because human cells are tested in a xenogeneic host, the question of histoincompatibility becomes a moot point since even malignant control cells would be rejected in inadequate immunosuppressive conditions.

Table 3 Assay for malignancy of human parent and hybrid cell populations

Cell lines	No. of cells inoculated into each mouse	No. of tumours/No. inoculated Tx + ATS*	T⁻B⁺†	Nude‡
D98/AH-2	8×10^5	5/6	10/10	6/6
HBU	8×10^5	6/6	9/10	6/6
Normal fibroblast	1×10^7	0/10	0/10	ND
ESH2	1×10^7	0/10	ND	0/6
ESH3	1×10^7	0/10	ND	0/6
ESH5	1×10^7	0/10	ND	0/6
ESH6	8×10^5	5/6	8/10	6/6

ND, Not done.

* Neonatal thymectomy plus antithymocyte serum.

† Thymectomy, whole-body irradiation followed by bone marrow reconstitution.

‡ Athymic nude mice, no immunosuppressive treatment.

Many other malignant–normal and malignant–malignant cell combinations must be tested before we can determine whether the suppression of malignancy reported here is a general phenomenon of human somatic cells If this is the case, it should lead to significant advances in the study of control of malignant expression in humans. It remains to be seen whether non-malignant hybrids derived from cancer cells retain the antigenic identity of their malignant parent. If so, they may be useful in cancer immunotherapy This possibility is strengthened by the fact that unlike mouse cells, human hybrids are chromosomally stable and seem to have their malignant phenotype completely suppressed.

I thank Maria Li and Mark Feinerman for technical assistance I thank Drs J. Schneider, E. Schneider, L. Hayflick and W Munyon for cell lines. These studies were supported by grants from the American Cancer Society and National Institutes of Health.

Received October 16, 1975; accepted January 16, 1976.

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Directed lipid flow in cell membranes

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A continuous and rapid oriented flow of lipid molecules in eukaryotic plasma membranes may explain capping of surface antigens.

MEMBRANES of eukaryotic cells exist in several discrete compartments[1]. These include the rough and smooth endoplasmic reticula, Golgi apparatus, the nuclear and plasma membranes and various cytoplasmic vesicles. How these membranes are related to one another is unclear, but structures inside the cell must be responsible for maintaining the high degree of organisation observed in cell sections. Any cellular features or processes which display a unique polar property may tell something about the basis for this organisation. One such process is the capping phenomenon described by Taylor *et al.*[2] in lymphocytes and observed by several groups in other cells[3–9].

Capping of antigens in the plasma membrane occurs when a particular surface antigen is cross linked, usually by specific antibody; the redistribution of these cross-linked antigens can be visualised using fluorescent antibodies. Two types of redistribution are found: the antigens form patches (which are just two-dimensional precipitates in the plane of the fluid membrane[9]) and are seen as discrete fluorescent areas on the cell surface. These patches may then form a cap, seen as a fluorescent crescent which in B lymphocytes occur at the end of the cell remote from the nucleus. Capping, unlike patching, requires metabolic energy and is thus an active process. In lymphocytes, where it has been most extensively studied, capping can be prevented by a combination of drugs which are believed to disrupt or inactivate microtubules and microfilaments—colchicine and cytochalasin B (ref. 10). Furthermore, once the cap has formed, addition of these drugs may enable it to disperse[10]. The main requirement, however, for molecules to be capped is that they be cross linked[2,7,11]; other antigens, or antigens labelled with Fab antibody fragments, do not patch, nor do they cap.

The mechanism of cap formation has remained unclear, although it is generally believed that the surface antigens, once cross linked from outside the cell, are moved to one pole of the cell by cytoplasmic structures which may include microtubules and/or microfilaments[12–15]. Interpretations of capping along these lines become difficult to understand when one tries to consider them at a molecular level. How do the cytoplasmic components recognise that surface antigens are cross linked? Any simple explanation would seem to require that the surface antigens span the cell bilayer, a situation for which there are several precedents[16–19]. There then exist at least two plausible mechanisms for capping: either cytoplasmic components recognise a "new" distribution of those ends of the surface antigens on the cytoplasmic side of the membrane, and somehow select these to be capped. This mechanism is hard to contemplate as the membrane is a liquid and these surface antigens must frequently come close to each other due to Brownian motion. Alternatively, surface antigens could all be allosteric proteins; when cross linked from outside the cell, a conformational change of that part of the antigen on the cytoplasmic side of the membrane could be recognised as "new" by cytoplasmic components, and lead to the selective movement of these molecules to the pole of the cell. There are several difficulties with this hypothesis: (1) Fab fragments which bind to surface antigens might be expected to cause a conformational change—yet capping does not result[2]; (2) apparently any surface antigen can be capped, which would imply a bewildering amount of recognition by cytoplasmic components; (3) lectins, which bind to sugar residues on surface glycoproteins, will cause capping of those molecules to which they bind[7,11], yet it seems unlikely that binding lectins to the carbohydrate portion of a protein would change its conformation.

Here I present a radically different interpretation of the capping process which may have interesting implications for other cellular processes.

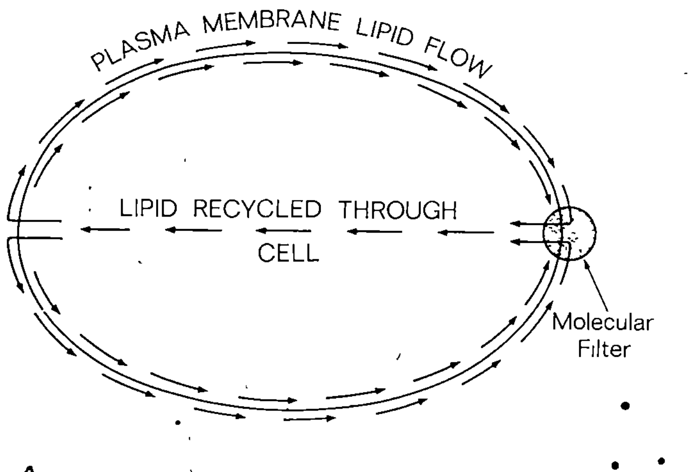
Continuous lipid flow in cell membranes

The hypothesis I wish to put forward is that there is a rapid, continual and oriented flow of lipid molecules in the plasma membranes of motile eukaryotic cells. Lipid molecules are inserted into the plasma membrane from the cytoplasm at certain sites on the cell and are resorbed back into the cytoplasm at different sites on the cell. This is shown schematically for a lymphocyte, in which there seems to be a lipid flow from one end of the cell to the other, in Fig. 1. Addition and removal of lipids presumably occurs through vesicles (see below). Other molecules associated with the plasma membrane do not cycle in this way: they remain approximately randomly distributed in the membrane under the influence of Brownian motion. The precise distribution of membrane proteins may not be quite random: the actual distribution will depend on many factors, including how fast the lipid flow is, the diffusion coefficient of the molecule in question and the length of the cell. But if a very large molecule is lodged in the plasma membrane, its rate of lateral diffusion may not overcome the lipid flow: it will then be swept to that site on the cell surface at which lipid resorption occurs. An example of such a large "molecule" is a set of cross-linked surface antigens. The individual antigens are no longer free to diffuse independently of one another and, if the patch is large enough, they will be carried along *en masse* by the lipid flow to the pole of the cell and will be seen as a cap.

Quantitative aspects of lipid flow

The idea that membranes flow is not new[20–23]. Ingram[24] and Abercrombie *et al.*[25] have observed the directed movement of large particles (such as carbon black) on the dorsal surface of fibroblasts from their ruffling edges to an area over their nuclei. This was interpreted as showing that the surface membrane of

Fig. 1 Schematic diagram of recycling of lipids in a lymphocyte



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these cells is flowing. The difference between their interpretation and my proposal is that I believe there is a net flow which is largely confined to lipid molecules—and not membrane recycling which includes all the membrane proteins. In the case of lymphocytes, it seems that once antigens have been capped, their reappearance on the cell surface takes a long time (compared with capping) and requires protein synthesis[26]. In other words, capping does not seem to result from the recycling of surface membrane as a whole: if rapid membrane recycling exists, it must be limited to lipid molecules. Presumably the same holds for fibroblasts.

The rate of movement of particles on fibroblasts has been measured and is about 3 μm min^{-1} (refs 22 and 25). If a similar rate of lipid flow exists on a lymphocyte, then the time taken to sweep a cross-linked antigen on to one half of the cell (which is about 10 μm in diameter) would be about 2 min. This is roughly the time at which the first caps are seen to form on lymphocytes[2], and suggests that the rate of lipid flow in lymphocytes and fibroblasts is similar.

If a lipid flow rate of 3 μm min^{-1} exists across a flat lipid bilayer sheet, we can estimate how this will affect the distribution of individual protein molecules lodged in the membrane. The rate of lateral diffusion of rhodopsin molecules in frog retinal disk membranes has been measured by Poo and Cone[27]: these proteins diffuse at a rate of about 10 μm in 1 min. If the viscosities in disk and plasma membranes are similar, the lateral diffusion of a protein the size of rhodopsin will effectively win out over a lipid flow of 3 μm min^{-1}. There will, however, be a slight difference in concentration of antigens at the two ends of the stream. For a one-dimensional case in an equilibrium situation, the concentrations of a protein (C_a, C_b; a is upstream of b) are given by $C_a/C_b = \exp(-FL/D)$, where F is the flow rate, L is the distance between points a and b in the direction of the stream and D is the diffusion coefficient of the protein. For a flow rate of 3 μm min^{-1}, a diffusion coefficient of 4×10^{-9} cm^2 s^{-1} (for rhodopsin in disk membranes at 20 °C, ref. 27) and L of 10 μm (roughly the length of a lymphocyte), we get $C_b/C_a \sim 3$. Of course, the lymphocyte is a roundish cell so that F cannot be uniform over its surface and the parameters taken for rhodopsin may not be applicable here, especially as the viscosity in the plasma membrane of a cell growing at 37 °C may be somewhat lower than in the frog retinal disk membrane[28]. It is therefore difficult to predict just how great C_b/C_a would be. But if the protein molecule were made much larger, its diffusion coefficient (which is not easily estimated[29]) would decrease and could thereby change C_b/C_a by a large factor because of the exponential relationship between D and C_b/C_a. In other words, as a protein aggregate grows in size, C_b/C_a may increase sufficiently that a patch would be seen to cap.

These calculations simply show that, given the rather crude assumptions made, a lipid flow of a few μm min^{-1} could explain the capping process. They also indicate that surface antigens, or single proteins embedded in the bilayer, might not be quite uniformly distributed over a cell. This might be visible as a non-uniform distribution of particles in the freeze-fracture plane of a large cell, such as a fibroblast.

Implications of lipid flow

The most obvious prediction this model makes is that lipid molecules will not stay in the plasma membrane for long periods. More usefully, it predicts that a surface antigen need not communicate in any way with the cytoplasm to be swept along and thus be capped. Thus, phospholipids or glycolipids—molecules which by their nature only extend half way through the bilayer—should be able to be capped. The most direct way to test this would be to insert a new glycolipid into the bilayer of a cell not possessing that antigenic determinant, and see if that can be capped. This experiment cannot be unambiguous at present since there is no known way of showing that the added glycolipid is indeed properly inserted into the host cell's bilayer, rather than adhering to surface protein. Nevertheless, when such an experiment is attempted[30], the added glycolipid does cap. A better experiment might be to see if antibodies which bind specifically to phospholipids[31] could lead to capping.

There then remain two additional questions I should like to consider. If only lipids are resorbed into the cell, how is this done without concomitant resorption of plasma membrane proteins? A molecular filter would seem to be needed—a special vesicle designed to exclude membrane proteins. The other question is, where do the lipids go after internalisation? If they are fed into the internal membrane pool—the Golgi apparatus or endoplasmic reticulum—how is it that plasma membranes contain high levels of cholesterol (approaching equimolarity with phospholipids), whereas those membranes within the cell contain a low level of cholesterol which is only a fraction of that found in the plasma membrane[32]. In other words, the hypothetical filter I have in mind would remove not only plasma membrane proteins but might also remove much of the cholesterol. A possible candidate for this is the "coated vesicle", which is found in many cell types[33–39] and contains only one major protein, "clathrin"[38,39]. These vesicles are believed to be responsible for resorption of plasma membrane in nerve endings after stimulation of the neuromuscular junction[40]. They contain a small lipid vesicle inside a lattice network of clathrin[38,39]; as the lipid vesicle buds inwards from the plasma membrane, clathrin can be seen to coat the vesicle[33–35,38] and in so doing, might clear away other proteins to give a fairly pure lipid vesicle inside its coat.

Microtubules and microfilaments seem to be implicated in capping and thus in the recycling of phospholipids. How they do so is unclear, although there are hints that one of the functions of microtubules may be to shunt vesicles around[41–44], and microfilaments seem to be needed for various membrane movements such as ruffling[45,46]. The present arguments imply that somehow these fibrillar elements are involved in the directed movement of membranes within the cell.

In conclusion the polar property displayed by many eukaryotic cells in the capping process or in the movement of particles adsorbed on their surfaces can be explained at the molecular level by an oriented and continuous flow of lipid molecules in the cells' plasma membranes. There must be a source and a sink for this lipid flow; the sink is likely to require a molecular filter which removes most of the membrane proteins and perhaps cholesterol from the internalised membrane. This specific resorption process may be achieved by coated vesicles.

I thank Dr Graeme Mitchison for assistance with some of the estimates made here.

Received October 15, 1975, accepted January 30, 1976.

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Jurassic igneous rocks of the Forties Field

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Middle Jurassic volcanic rocks unexpectedly encountered during North Sea drilling operations seem to have oceanic affinities. Though this finding may seem surprising in a suite situated in a continental basin, it can be explained using a plate tectonic model. According to the model, the volcanism developed along a line of incipient sub-crustal rifting during the early opening of the Atlantic. Before crustal splitting occurred, however, the spreading axis migrated westwards and the well documented volcanism of the North-west Tertiary Igneous Province developed.

DRILLING operations in the Forties and Piper areas of the North Sea have unexpectedly encountered basic igneous rocks which seem to be part of a thick volcanic sequence of Middle Jurassic age[1]. As such, they represent a new and previously unsuspected igneous province in north-western Europe. The lavas, which have a maximum thickness of over 740 m, are porphyritic olivine basalts which "tend towards an alkaline affinity"[1]. We report here on the whole-rock geochemistry of the lavas; our preliminary findings are based on material from the Forties Field wells 21/9–1 and 21/10–1.

The material from well 21/9–1 represents a 17-m vertical section comprising (in descending order):

(1) The lowermost 0.65 m of a porphyritic basalt flow (the whole of which is probably not much thicker).
(2) A 2.75-m thick flow of porphyritic vesicular basalt with a brecciated upper surface.
(3) 12.6 m of porphyritic basalt which could be a single flow or as many as three separate flows.
(4) The top metre of a porphyritic vesicular basalt flow of indeterminate thickness.

The section represented by the material from well 21/10–1 is comparatively short, comprising 1 m of porphyritic basalt on top of 0.5 m of porphyritic vesicular basalt, which in turn lies on top of sediments. The change from non- or sparsely vesicular basalt to markedly vesicular basalt may correspond to the junction between two flows, but the exact relationship is obscured by the altered nature of the rocks and the fact that just above the transition the porphyritic basalt is intruded by a 0.5-m thick sheet of vesicular 'dolerite' with chilled upper and lower margins.

Petrography

All of the flows represented in the well 21/9–1 section contain abundant, large phenocrysts of clinopyroxene (Cpx) and pseudomorphs after olivine (Ol). Phenocrysts of plagioclase are present in varying amounts but are much smaller. The pyroxene is virtually unaltered, but the olivine is almost entirely replaced by serpentine, chlorite and carbonate and often considerable amounts of iron oxide. The lavas from well 21/10–1 also contain phenocrysts of clinopyroxene and pseudomorphs after olivine, but differ from those of well 21/9–1 in apparently lacking the plagioclase microphenocrysts and in containing abundant basaltic hornblende. The pyroxene phenocrysts in the lavas from both wells vary in size, but can be a few centimetres across, and exhibit spectacular, discontinuous, oscillatory and sectoral zoning. The mineral chemistry of the pyroxenes is still under investigation, but it is evident from their optical properties that they are augites with significant contents of Al and Ti, and are thus typical of the phenocrysts found in basic igneous rocks of alkaline affinity. This, together with the obviously high original olivine content, led Howitt *et al.*[1] to deduce that the rocks were probably alkali olivine basalts.

The groundmass of all the lavas is fine grained and very altered, but small plagioclase laths or microlites, minute augite crystals and magnetite/haematite grains can be discerned among the chlorite and other alteration products. As is typical of alkaline basaltic rocks[2,3], all of the specimens are more or less altered, particularly with regard to oxidation, which is most marked towards the tops of the flows. The top of the thickest flow in the well 21/9–1 section is so oxidised that it consists almost entirely of haematite. This red 'bole' is suggestive of subaerial weathering and seems to be incompatible with suggestions[4] that the volcanics were subaqueous.

Although the indications are that the North Sea igneous rocks are of alkaline affinity it is difficult to be more precise on petrographic grounds, largely because of their altered nature, so we have investigated the whole-rock geochemistry. A number of samples from each of the flows represented in the two well sections have been analysed for major and trace elements (by a combination of X-ray fluorescence (XRF) and chemical methods) in order to study the systematic chemical variations which exist within and between individual flows (Table 1). More detailed geochemistry will be described elsewhere.

Whole-rock geochemistry

The classification of volcanic rocks on the basis of normative mineralogy becomes increasingly difficult and unsatisfactory as the rocks become more altered. Various methods have been suggested to overcome the effects of alteration, especially oxidation[5–8]. We have followed Thompson *et al.*[9] in reducing the Fe_2O_3 content to 1.5% or 2.0% (for total alkali contents of less than 4% and less than 7%, respectively) before calculating the norm. The representative analyses presented in Table 1 consist of the average compositions of two of the thickest flows from well 21/9–1 (9B, 9C), a typical specimen of lava from well 21/10–1 (11211) and the two samples from well 21/9–1 with the highest and lowest K_2O contents of those so far determined (3395.25, and 3396.5). All are nepheline normative except for the sample with the lowest K_2O content from well 21/9–1, which is only just hypersthene normative. Though this is consistent with alkali olivine basalt affinity, the altered state of the rocks does not permit unequivocal interpretation.

Another method of identifying the parentage of basic

Table 1 Analyses and CIPW norms of North Sea volcanic rocks

Number		9.C	9.B	11211	3395.25	3396 5
SiO_2		44.61	43.96	44.27	44.20	43.59
TiO_2		2.78	3.12	2.47	2.87	2.99
Al_2O_3		12 84	13.95	9.72	12.51	13.39
Fe_2O_3		6.91	7.13	4.44	6.09	8.85
FeO		4.45	4.53	5.84	5.43	3.46
MnO		0.22	0.22	0.20	0.40	0.14
MgO		10.60	9.18	14.27	11.26	8.66
CaO		9.84	8.40	13.01	12.13	7.07
Na_2O		2.36	2.61	1.48	1.75	3.13
K_2O		1.05	1.61	0 71	0 45	2.35
P_2O_5		0.47	0.52	0.40	0.45	0.50
S			0.01	0.06		
H_2O^+		3.55	4.47	2.87	3.06	5.52
CO_2		0.22	0.28	0.22		0 28
Total		99.90	99.99	99.96	100 60	99.93
Or		6.21	9.52	4.20	2.66	13.89
Ab		18.37	19.40	9.17	14.81	17.77
An		21.34	21.60	17.78	24.95	15 55
Cpx	Wo	10.19	6.97	17.85	13.48	6.04
	En	6 65	4.52	12.68	8.87	3.76
	Fs	2.84	1.97	3.62	3.66	1.93
Opx	En				0.08	
	Fs				0 03	
Ol	Fo	13.84	12.86	16 02	13.38	12.48
	Fa	6.52	6.18	5.04	6.09	7.06
Ne		0.87	1 46	1.82		4.72
Mt		2.18	2.90	2.18	2.18	2.90
Il		5.28	5.93	4.69	5.45	5.68
Ap		1.09	1.21	0.93	1.05	1.16
Py			0.02	0.11		
Cc		0.50	0.64	0.50		0.64

volcanics is to plot them on a total alkalis against SiO_2 diagram (Fig. 1). It is evident that on this criterion the North Sea volcanics are, on the whole, mildly alkaline, with a few more strongly alkaline varieties.

The problems of correctly classifying altered volcanic rocks, especially alkaline types, has been discussed by a number of authors[3,10,11], particularly with regard to identifying their tectonic setting. Probably the most satisfactory method of typifying the North Sea basalts is the TiO_2 against Zr/P_2O_5 plot of Floyd and Winchester[11] which uses 'immobile' elements, thus minimising the effects of any subsequent alteration of the rocks. The North Sea lavas (Fig. 2) fall within the fields of both continental and oceanic alkali basalts and lie outside the fields of tholeiitic basalts. In addition, the suite exhibits the vertical trend characteristic[11] of alkali basalts and atypical of saturated volcanics. Trace element contents, particularly Sr and Rb are also consistent with alkali basalt parentage. The Sr and Rb contents are of the order of 600 p.p.m. and 20 p.p.m. respectively. Typical tholeiites contain less than 150 p.p.m. Sr and only a few p.p.m. Rb, whereas alkali basalts can contain up to 1,000 p.p.m. Sr and 100 p.p.m. Rb (refs 12 and 13). It therefore seems clear that the analysed Jurassic lavas from the Forties Field are mildly undersaturated rocks of alkali olivine basalt parentage.

Geochemical discrimination between alkali basalts from continental and oceanic tectonic settings is notoriously difficult (see Carmichael *et al.*[13]). One possible method is to use the TiO_2–K_2O–P_2O_5 ternary plot proposed by Pearce *et al.*[14]; but attempts to apply this method result in the analyses plotting along the boundary between the two types. Direct comparison of the North Sea lavas with other alkali basalts in terms of major elements reveals that individual analyses can be matched to a remarkable degree with basalts from such diverse tectonic settings as the East African Rift Valley[15,16] and Hawaii[17]. Surprisingly, in view of the continental nature of the North Sea crust, the lavas seem to have more in common with oceanic alkali basalts than with those from continental settings. Indeed, in many geochemical respects, the alkali basalts from Hawaii seem to be the most directly comparable with those from the North Sea. With regard to this occurrence of alkali olivine basalts apparently having oceanic affinities within a continental plate, it is worth considering the structure of the North Sea Basin at the time of the volcanism and its possible relationship to the large scale tectonic pattern.

Plate tectonic development

During the Jurassic the North Sea area was dominated by a series of major troughs, or graben. The volcanic rocks occur close to the confluence of three of these—the Witch Ground, Viking and Central Graben[1]. It has been suggested[18] that this trough system may have originated in Hercynian times but if so there seems little doubt that it was being reactivated during the Jurassic[4]. Tectonically, therefore, the best analogy for the Jurassic North Sea may be the East African Rift Valley where a comparable rift system is developing on continental crust, and the similarities between individual volcanic rocks of the two areas lends some support to this possibility.

The general north–south rifting in the North Sea area during the Jurassic could be connected with the subduction of the Tethys plate below the Russian Platform far to the south-east[4], or, alternatively, it could simply be a result of the stress system developed at continental margins because of differences in crustal density[19]. Neither, however, could account for the thinning[18] of the continental crust, or the occurrence of the igneous rocks, both of which are consistent with high heat flow in the underlying asthenosphere. Whiteman *et al.*[18] have suggested that the North Sea rift systems may be failed arms of plume-generated rrr trilete junctions, but they attribute the rift system in the Forties area to uplift caused by mantle plume activity during the late Carboniferous and early Permian. Consequently, though mantle plume activity could certainly account for igneous activity, the timing is inconsistent with the Middle Jurassic age of the North Sea volcanic rocks and, in any case, there seems to be little evidence other than the rift systems to support the suggestion that plume activity occurred beneath the North Sea area from the Carboniferous to the Cainozoic.

We suggest the following model based on a general consideration of Laurasian global tectonics at the time of volcanism.

During the Lower Jurassic (approximately 180 Myr BP) the Atlantic began to open south of the Azores[20,21]. The spreading axis is generally regarded as being truncated by a transform plate margin running from south of Spain to south of Newfoundland. By the beginning of the Cretaceous the mid-Atlantic spreading axis extended much further north, "possibly as far as the western approaches to the English Channel"[21], and it seems reasonable to presume that this northward extension existed in an embryonic form during the Jurassic. When it matured into

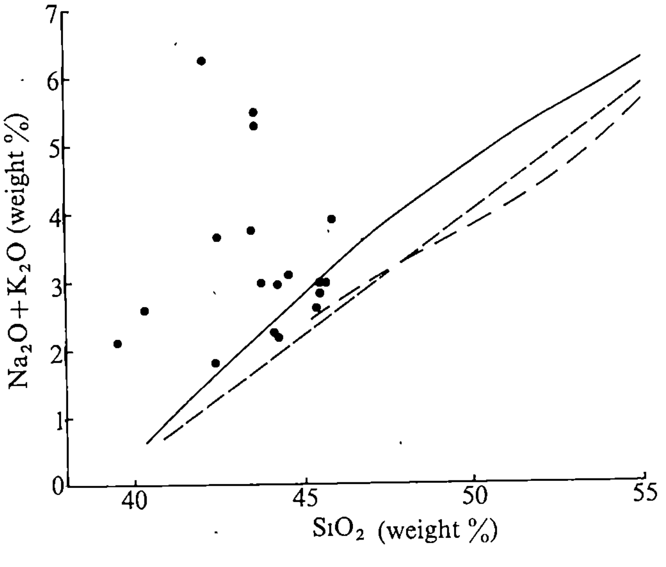

Fig. 1 Total alkalis against SiO_2 plot for North Sea volcanic rocks. Lines denote the division between alkaline and sub-alkaline types: ———, after Irvine and Baragar[6]; — — — —, after Macdonald and Katsura[17]; — · — · —, after Kuno[2].

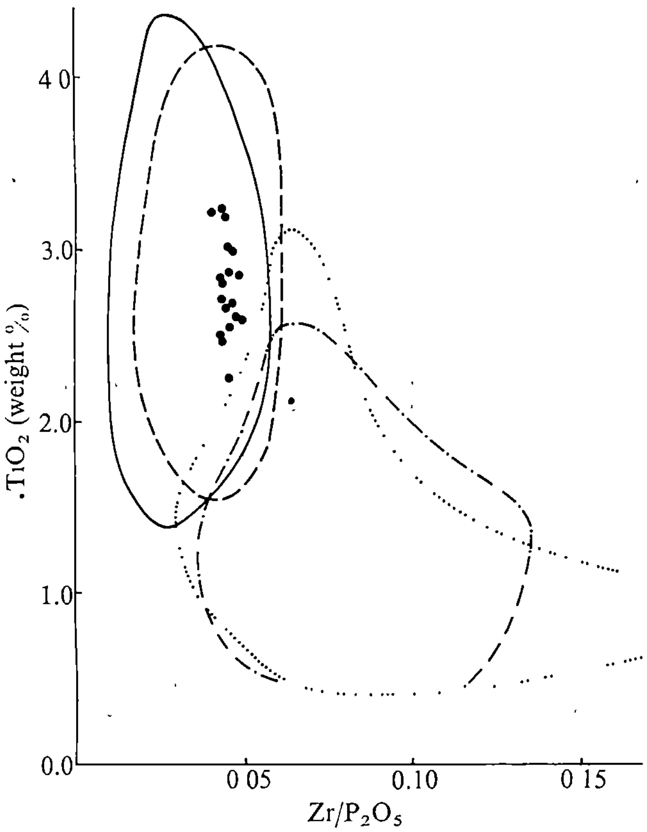

Fig. 2 TiO_2 against Zr/P_2O_5 plot for North Sea volcanic rocks. Field boundaries after Floyd and Winchester[11]: ———, continental alkali basalts; — — — —, oceanic alkali basalts; · · · ·, continental tholeiitic basalts; — · — . —, oceanic tholeiitic basalts.

an active spreading axis this northward extension could have terminated[21] at the edge of the continental crust against the Bay of Biscay–Labrador Sea transform fault. It is generally believed that no spreading axis existed north of this transform fault at least until the late Cretaceous, when it emerged west of the UK continental shelf and offset along the transform fault. If the embryonic spreading axis (or the underlying feature which gave rise to it) to the west of Spain in Jurassic times were projected northwards below the continental crust, it would pass underneath the North Sea, and it could be significant that the North Sea volcanic rocks occur at the intersection of this extension with the Fair Isle–Elbe wrench fault[4]. Since any offset along the Bay of Biscay–Labrador Sea transform fault would be insignificant until new oceanic crust began to be generated by the mature spreading axis, such a linear projection does not seem unreasonable. What is proposed, therefore, is that from its inception during, or possibly before, the Middle Jurassic, the spreading axis north of the Azores (or its underlying parent feature) extended northwards beneath the continental crust at least as far as the North Sea, and possibly as far as the hinge point for the opening of the central Atlantic which was situated in south Norway[20,21]. Activity along this northward extension was insufficient to produce a splitting of the crust but enough to contribute to an approximately east–west tensional regime with consequent thinning of the crust and reactivation of rifting in the North Sea area. Though little or no new crust was generated along this extension (the spreading axis was only just becoming established west of Spain), the uprise of heat in the asthenosphere below the North Sea could have generated the volcanic rocks by the partial fusion of the mantle, with the ensuing magma reaching the surface through the thinned crust along lines of structural weakness—the Fair Isle–Elbe line in this particular case. As the Atlantic continued to spread, the continental crust underlying the British Isles was dragged eastwards by the Eurasian plate as a trailing plate, and the extension of the spreading axis below the continental crust underwent a relative westward migration, becoming more and more offset along the Bay of Biscay–Labrador Sea transform fault. During the Cretaceous this westward migration reached the eastern edge of the Rockall Trough. By the time of Anomaly 24, the migration had moved the spreading axis to the west of the Rockall Bank and the well documented opening of the North Atlantic between Greenland and Europe began.

Our model, though admittedly speculative, involves a deep-seated feature of the type generally associated with the genesis of oceanic volcanic rocks, and that, together with the thinned overlying continental crust, could conceivably account for the nature of the North Sea volcanic rocks. The model is also consistent with a tensional regime in the Jurassic North Sea Basin and its subsequent transition to a compressional regime during the late Cretaceous[4].

We are grateful to British Petroleum Limited for making material available for study, and to Dr F. Howitt for his comments.

Received November 19, 1975; accepted January 28, 1976.

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letters to nature

Direct observation of pulsar microstructure

HANKINS' observations[1] of pulsars exhibiting structure on time scales as short as a few μs have set stringent requirements on the theories which attempt to explain the pulsar emission mechanism[2,3]. These microstructure observations[1,4,5] were made at low radio frequencies where pulsar intensities are high. To overcome the effects of interstellar dispersion, which effectively 'smear out' the microstructure, Hankins developed a complex computation method. Since the effects of dispersion are less pronounced at higher frequencies, microstructure should be directly observable

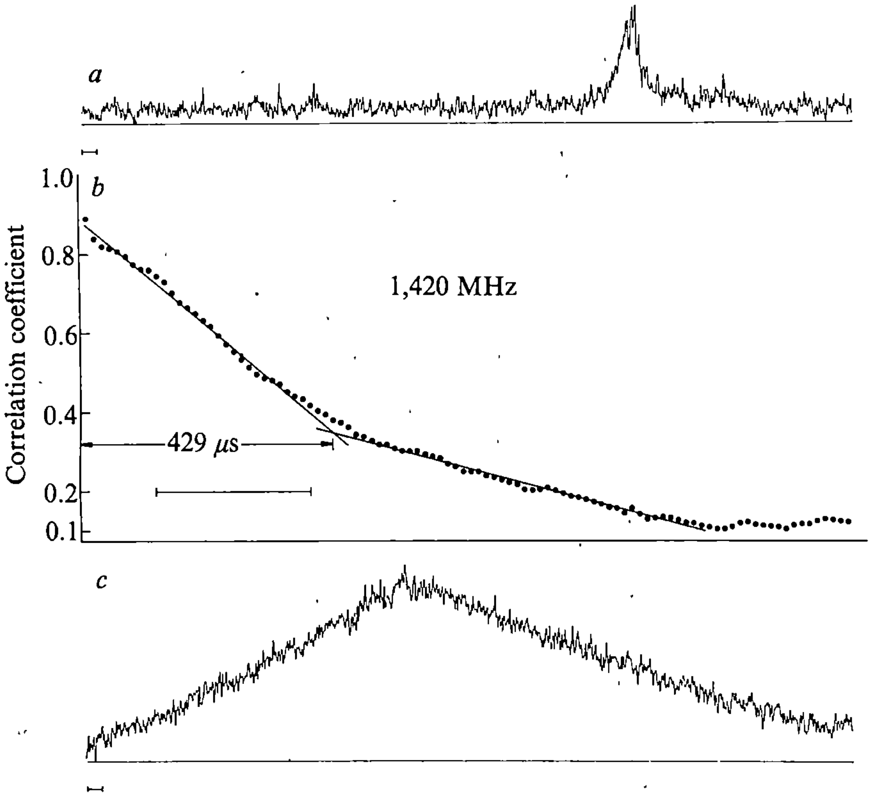

Fig. 1 *a*, A single pulse from PSR1133+16 with a time resolution of 14 μs; *b*, its autocorrelation function; *c*, the associated integrated pulse shape obtained by adding 2,099 pulses. In all of our figures, the time scale is indicated by the 260-μs bars underneath.

using an appropriate bandwidth This letter reports high-frequency observations of PSR1133+16 where the dispersion broadening and the time resolution were short enough to reveal microstructure on a time scale of 14 μs.

We used the 100-m radiotelescope of the Max-Planck-Institut fur Radioastronomie at Effelsberg[6] at an observing frequency of 1,420 MHz, with beamwidth 9′ and antenna performance 1.5 K Jy^{-1} The signal was detected with a linearly polarised feed at the prime focus The first stage of the receiving system was a cooled parametric amplifier giving an overall system noise temperature between 50 K and 65 K, depending on elevation. For pulsar microstructure studies, various filters at the intermediate frequency were available to allow the correct bandwidth to be chosen for the optimum resolution for each pulsar. After detection the signal was digitised by a voltage–frequency converter (with a maximum output frequency of 15 MHz), in conjunction with the pulsar data logger (PDL) This device enables the storage of 1,024 numbers per pulse period, and is controlled by two computers The first, a Ferranti Argus 500, provides Doppler-corrected pulsar period and timing signals. The second, a Honeywell H316, takes the data or buffer storage and transfers them through the Argus 500 computer on to magnetic tape.

A number of pulsars were recorded with various resolutions on November 17 and 25, 1975. Observations of the pulsar PSR1133+16 were made with a resolution of 13 μs. The bandwidth used was 1 MHz, which in view of the pulsar DM of 4.85 pc cm^{-3}, resulted in a dispersion broadening of 14 μs. The data from each pulse were recorded using 1,000 resolution intervals, each 13 μs long. Most of the observations were restricted to the first component of the double-pulse profile.

Our results for both components of PSR1133+16 confirm the general features of the microstructure as described by Hankins[1,4]. Autocorrelation functions (ACFs) of the strong pulses often show the ‘linear’ feature associated with microstructure, extending from near zero lag to a few hundred μs. This is followed by a much broader feature associated with the subpulse width Many pulses lack the microstructure feature in their ACFs which seems to imply either that microstructure is not always an important part of the

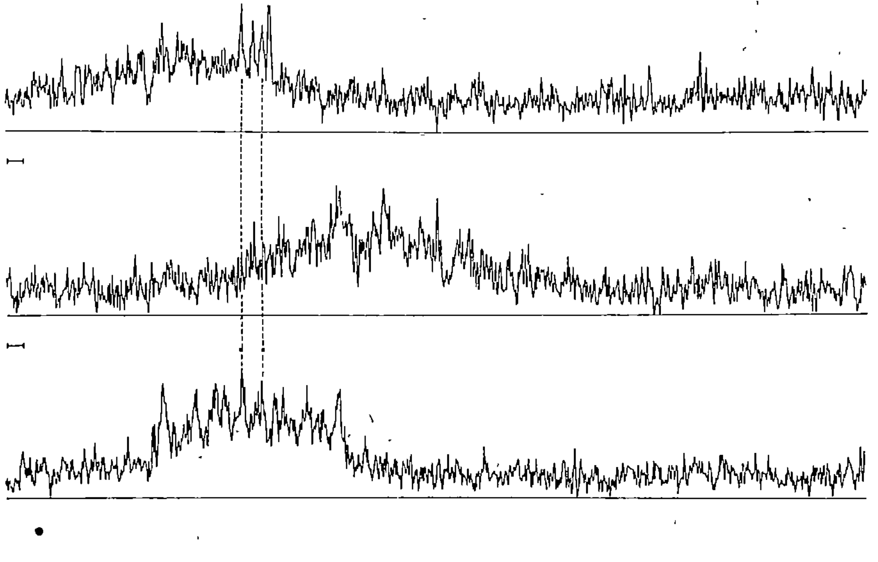

Fig. 2 Three consecutive pulses from PSR1133+16 showing a quasi-periodic behaviour within the subpulses.

emission, or that, if it is present, it is sometimes totally unresolved at 14-μs resolution. Spikes which are comparable in width with our time resolution are rare, however, and seem to be relatively weaker than those reported at 111.5 MHz by Hankins and Cordes[5]. The flux intensity of the strong pulses in our observations reaches a maximum of 200 Jy.

A pulse (taken from the first component of the integrated profile), which shows microstructure, its ACF and the integrated pulse shape obtained from it and 2,099 surrounding pulses are shown in Fig. 1*a*, *b* and *c* respectively.

An interesting feature which appears in some pulses is a quasi-periodic behaviour within the subpulse. Three consecutive pulses showing this behaviour in their ACFs are shown in Fig. 2. The apparent periodicity is 150 μs. Hankins remarked that similar behaviour sometimes appears in PSR0950+08, but to our knowledge it has never before been reported for PSR1133+16. Furthermore, the fact that some peaks occur at the same phase in the two strongest pulses, which have subpulse peaks at different phases, makes it uniquely interesting. Of course, this quasi-periodicity appears in the ACFs as secondary maxima at the lags corresponding to the 'period' and its multiples.

Studies of these observations of PSR1133+16, as well as 20-μs resolution observations already obtained for PSR0950+08 and observations of several other pulsars at resolutions <200 μs are still in progress and should yield further information on what is the basic pulsar emission process.

We thank all of the people who made the PDL a reality, in particular U. Reith and H. Hutfless.

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Received December 22, 1975, accepted January 26, 1976.

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A possible large-scale meridional structure in the helioequatorial solar wind

OVER the past few years observational data for a large-scale meridional structure of the solar wind have been accumulated[1-3]. Space-probe measurements have revealed heliolatitudinal effects at 1 AU, but little is known about the wind flow pattern at small radial distances, just outside the corona. Here we report the existence of a solar equatorial belt, from which few protons are emitted, and we discuss its causes.

We studied the semi-annual variations in solar cosmic ray propagation with the change of heliolatitude of the Earth. The probability of the arrival of solar cosmic rays at the Earth may be used to measure the conditions for the propagation of solar particles in space, between the solar flare and the Earth. This probability is proportional to the ratio of the proton flux (E_p > 10 MeV) to the energy of a solar radio burst (2,800–9,400-MHz range)[4]. A list of proton flares[4] was used to calculate this ratio averaged over 30 d. In Fig. 1*a* the probability *K*, determined in this way, as a function of the Earth's heliolatitude for 1957–63 is presented (182 proton events). One can see that very few solar protons penetrate a certain 'layer' centred on the solar equatorial plane (~ ±2° of heliolatitude). This avoidance ('quiet') zone in the solar particles propagation was found also in the data for 1938–56 and 1965–69. The same effect is seen when events in the northern and the southern hemispheres are analysed separately. Clearly the avoidance zone does not completely 'isolate' one solar hemisphere from the other, so the particles emitted by flares in the northern hemisphere can penetrate into the southern region and vice versa, but the mean flux of solar protons decreases when particles cross the equatorial plane (for details see ref. 5).

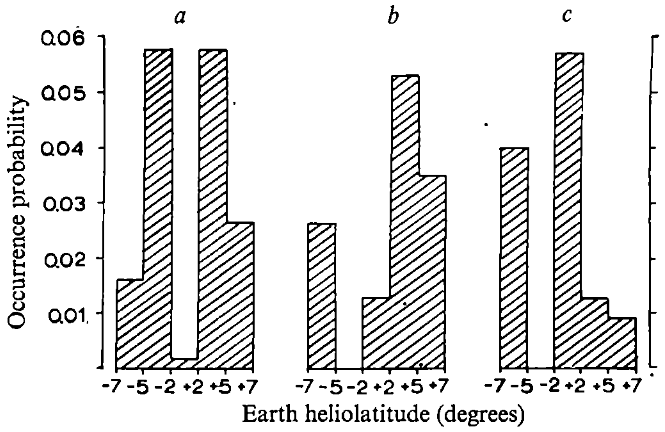
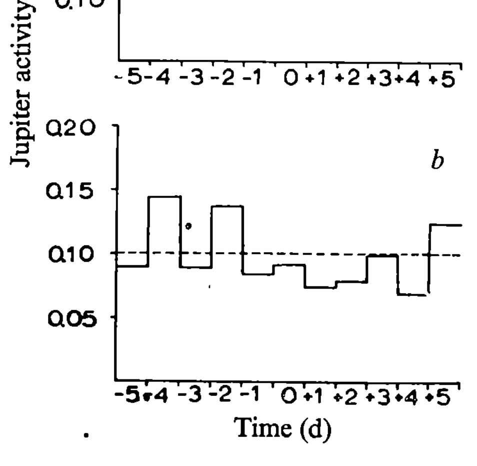


Fig. 1 *a*, The probability of the occurrence of solar cosmic rays at the Earth, *K*, as a function of the Earth's heliolatitude (1957–63). *b* and *c*, The displacement of the avoidance zone of solar cosmic rays in 1965–69; *b*, Events in the northern hemisphere; *c*, Events in the southern hemisphere.

The quiet zone effect was also revealed in the influence of proton flares on Jupiter's decametric radio-burst activity. Some evidence for an association of the period of Jovian activity with the solar type-IV radio bursts was found by Warwick[6]. This correlation was not, however, confirmed later[7,8]. Data from Boulder[9-12] were studied again to search for the effect of solar

Fig. 2 Decametric radio-burst activity of Jupiter after the proton flares (using the superposed epoch method). The ordinate gives the mean indices of Jovian activity. *a*, When the heliolatitude of Jupiter > 2°; *b*, when Jupiter is within the avoidance zone (heliolatitude < 2°).



protons on noise from Jupiter for 1960–71. The indices of Jovian activity were calculated for each day of a 4.5-month period near each opposition (for the indices we adopted the ratio of duration of the storm to the full time of the observation, with weight equal to the importance of the storm). The days when solar proton events as well as solar flares with a type-IV radio burst were observed, were taken as the zero days for the superposed epoch method. Two time intervals were considered separately: first, when the heliolatitude of Jupiter is $> 2^\circ$ and second when this latitude is $< 2^\circ$. It is evident from Fig. 2*a* that Jovian burst activity increases after flares with a delay of 1 d. The effect exceeds 5σ only when Jupiter's heliolatitude is $> 2^\circ$ (see Fig. 2*b*). Thus the avoidance zone probably exists at distances up to 5 AU (ref. 13).

These results suggest that a 'screen' might exist in the solar equatorial plane, probably a region of increased interplanetary magnetic field. It is possible that the particle density increases in the same layer. Such a regularity in the meridional distribution of the particle density near the Sun ($\gtrsim 5\ R_\odot$) has been inferred[14,15]. Some density excess in the equatorial plane may, however, be expected also at 1 AU. A density excess of 20–30% has perhaps been seen by Hundhausen *et al.*[16]. A corresponding decrease of the plasma velocity in the equatorial plane was revealed by the same measurements[16] in agreement with early results[14] and recent cometary data[17]. Some heliolatitudinal changes were also observed in the interplanetary magnetic field (for example in refs 18–20).

Out study therefore strongly suggests that the solar equatorial wind has a considerable meridional structure near the Sun: an annular region of quasi-stationary plasma with an increased magnetic-field intensity may be assumed in the equatorial plane. The angular width of this layer is $\sim 3^\circ$–4° (as it is seen from the Earth). The radial distance from the Sun is $\gtrsim 5\ R_\odot$. This hypothesis is in good agreement with calculations of the dynamics of solar wind expansion[21–24], which predict the existence of a meridional component of the solar wind velocity directed towards the equator at a distance of several $R_\odot$ (inside the critical Alfvén point). The magnetic field lines would be squashed into the equatorial plane (see also ref 25).

The displacement of the observed structure to the south ($\sim 3^\circ$) for 1965–67 was revealed by the finding of a very large N–S asymmetry in the distribution of solar activity[26]. A possible displacement of the lines of wind flow in such an asymmetry was suggested in ref. 27. The location of the avoidance zone is shown in Fig. 1*b* and *c* (for details see ref. 28). The result obtained agrees with the findings of other authors[15,29,30] and with the calculations[33,34] based on the space-probe measurements[31,32].

The authors are very grateful to A. B. Severny for discussions.

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Received June 30, 1975; accepted January 5, 1976.

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Solar constant during a glaciation

THE causes of ice ages are of great topical and scientific interest. Here we review some recent work on the subject, point out some severe objections to postulated mechanisms, and make our own proposal that the driving force is some periodic behaviour in the Sun's interior.

Kukla[1] has revived the Milankovich mechanism for glaciation, and values of the solar constant at the autumnal equinox suggest that the climatic optimum of 6,000 yr ago may be accounted for by a run of warm autumns. If the Milankovitch curve[2,3] is compared with the climatic factors obtainable from magnetically dated deep-sea cores, however, it is clear that this mechanism cannot cause the major glacial cycles (see Fig. 1 and refs 4 and 5) The major variations of the aridity of the land north of Dakar, the trade wind vigour and the ^{18}O glacial stages are in good correlation. There are ~ 8 such cycles down to the 0 7-Myr magnetic date, but, in the Milankovitch curve there are 15 occasions in this period when the summertime radiation at 65°N is significantly below average. At best, the Milankovitch mechanism may be responsible for the minor ripples superimposed on the major trends.

An attractive theory of glacial cycles has recently been proffered by McCrea[6]. He supposes that the Sun passes through a series of very dense dust clouds which border the concave edge of the galactic spiral arms. In a cloud, the Sun accretes matter and its luminosity could increase to high values. McCrea relates these 50,000-yr periods to the glacial stages, supporting Simpson's hypothesis that the warmer Sun provides sufficient precipitation to support a glaciation[7] This must, however, be viewed with suspicion because it is now established that temperatures fell during a glaciation, in the tropics and at the poles Furthermore, geological evidence[8] shows that centres of glaciation were the Scandinavian highlands, the Labrador plateau and other moist mountainous regions. Today these regions receive copious precipitation, yet no continental ice caps grow; probably because they are not cold enough. The McCrea mechanism is essentially one of random occurrence; yet, particularly from core evidence, the climate seems cyclical —crudely sinusoidal, with perhaps systematically increasing amplitude and period for the later cycles[4,5].

I suggest that some solar periodicity is the prime cause of the major climatic cycles, and try here to estimate (using Houghton's radiation curves[9]) the Earth's radiation and heat budget that would be in balance with a glacial climate This leads to a new value of the solar constant which I assume relates to the luminosity of the Sun.

It is difficult to calculate glacial temperatures Wilson and Hendy[10] have reviewed the ^{18}O data and believe that if the equatorial regions fell by 5 °C the polar regions would fall by ~ 14 °C. The extensive work on foraminifera in Atlantic cores by the CLIMAP group[11] shows that 18,000 yr ago the surface waters, from Newfoundland to the Bay of Biscay were colder by 10 °C and that most of the tropical waters were colder by 4 °C. Botanical studies in Europe[12] have established the July 10.5 °C isotherm during the Würm glaciation as running in the general direction

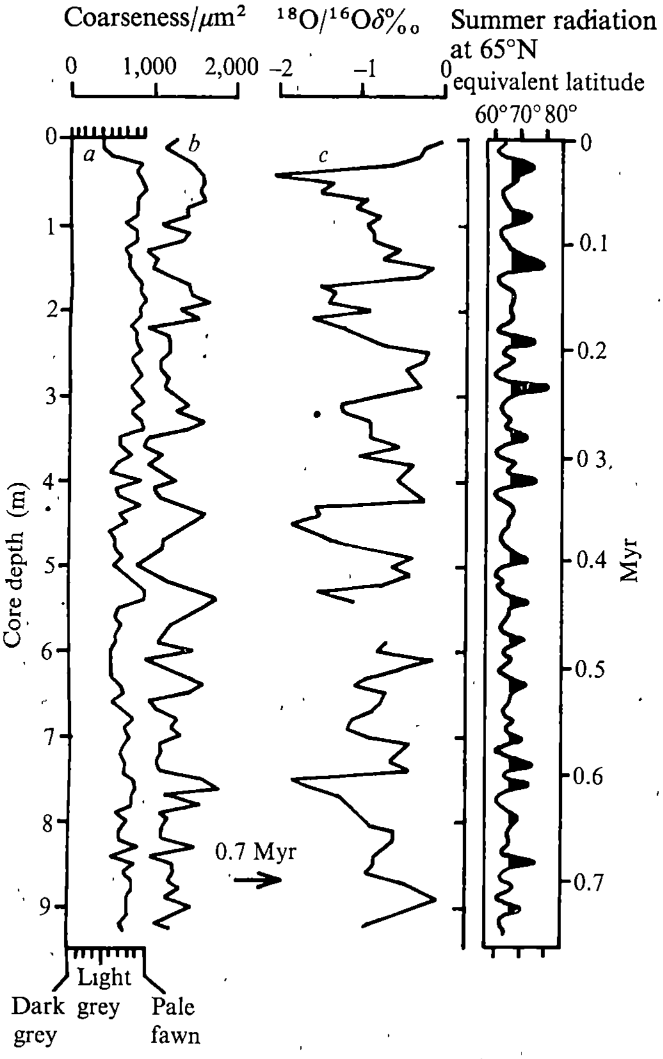


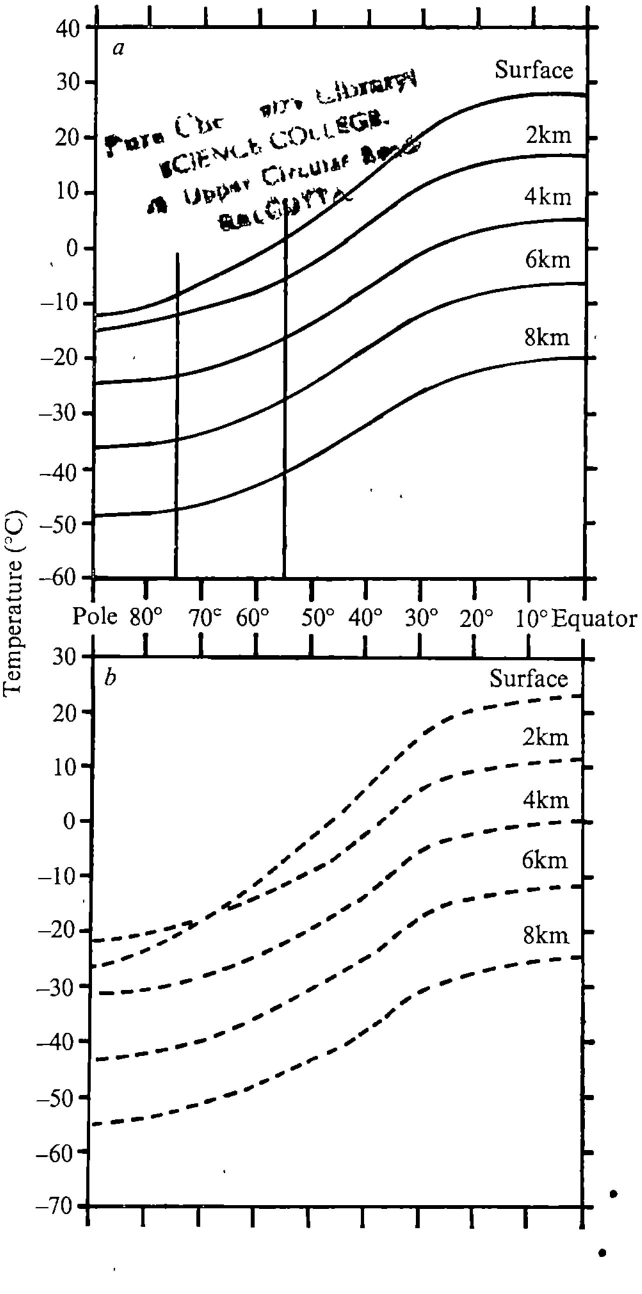
Fig. 1 The comparison between the climate factors down core V23-100 (from 21°18′N, 22°41′W, off the Sahara coast, just north of the Cape Verde Is.) and the Milankovitch curve. *a*, The colour is a measure of vegetation on the Spanish Sahara. The general lack of greyness means that humus (or burnt vegetation) was absent in the soil, that is, desert conditions prevailed throughout the whole time span. *b*, The coarseness is a measure of the size distribution of quartz particles A high value means strong trade winds, capable of transporting large particles. *c*, The ^{18}O deviations from present-day values measure the ice volume and establish the glacial stages. Note that strong winds and a slight increase in aridity occur during glacials.

Bordeaux–Berlin–Warsaw–Moscow. Today this line is the 18.5 °C July isotherm. By determining the degree of racemisation of aspartic acid in fossil bones, Schroeder and Bada[13] have estimated falls of 4 °C for the Mediterranean coast and 5–6 °C for East Africa. Judging drops in temperature from the lowering of the snow line is uncertain, as it depends markedly on precipitation; but assuming no change for Mount Kilimanjaro then an ~ 5 °C fall[8] would be reasonable for equatorial mid-tropospheric air. In estimating the long-wave radiation back to space, I generalise these data and assume surface level falls of 14 °C polewards of 80°N, 11 °C between 80°N and 60°N, 8 °C between 60°N and 40°N, and 5 °C from 40°N to the Equator.

In Fig. 2*a*, annual temperature profiles are shown at regular levels in the troposphere. The present-day ice cap is taken at a mean latitude of 75°N and the glacial ice cap at a mean latitude of 55°N (see ref. 7). This itself suggests a surface fall of 11 °C ~70°N, but a fall of only 6 °C for the mid-tropospheric air. In Fig. 3*a*, Houghton's annual absorbed solar radiations and re-emitted infrared radiations are reproduced (continuous lines), and the total heat flux across the circles of latitude are shown in Fig. 3*b*. Plotting the infrared radiation against the surface level temperature profile (Fig. 4), a linear relationship is obtained from the pole to ~ 20°N; however, against the upper air temperatures (say at 4 km) the empirical relationship is more complex. In the doldrum belt, towering cumulus cloud probably accounts for the 10°N and equatorial points plotting below the straight line. To estimate the infrared radiation to space during a glaciation, I assume that the present-day latitude points can be moved, parallel to the empirical line, by the appropriate temperature fall assumed for that latitude. This will tend to include the effects of the temperature-dependent changes in the concentration of water vapour[14]. The glacial infrared radiations are transferred to Fig. 3*a* as the dashed line.

Before the solar radiation curve can be proportionally reduced to secure a balance, it must be corrected for obvious changes in the planetary albedo. The present-day albedo (the inset in Fig. 3*a*) is redrawn along the dashed curve 1. This assumes that the glacial albedo at 55°N is the

Fig. 2 *a*, Present-day temperature profiles, constructed from mean meridional cross sections (Arctic Forecast Guide, US Navy Weather Research Facility, April 1962). The vertical lines give the position of the ice caps. *b*, Glacial temperature profiles. The surface level is based on currently available observational data; whereas the upper levels depend on the reliability of the method displayed in Fig. 4.



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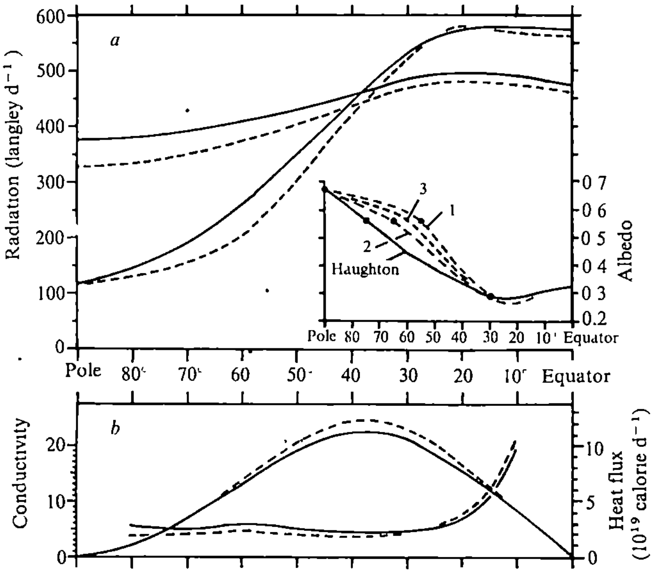
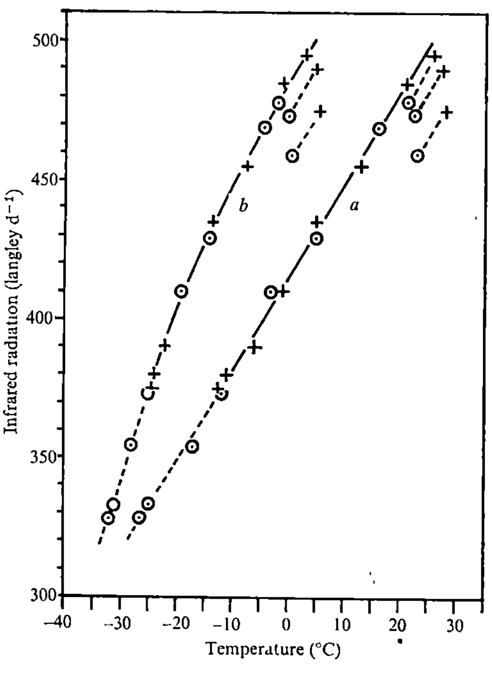


Fig. 3 *a*, The Houghton radiation curves, and the estimated radiation curves that are in balance during a glaciation for a 2.5% reduction in the solar constant. The inset shows the Houghton planetary albedo and the three glacial albedos discussed in the text. *b*, The Houghton heat flux and the estimated glacial heat flux across the circles of latitude. The latitudinal conductivity is also shown. Continuous lines refer to present-day values, dashed lines to glacial values. (1 langley = 1 g calorie cm^{-2}). The latitudinal thermal conductivity = (heat flux per cm length of the latitude circle)/(thermal gradient, as °C per degree latitude).

same as that now at 75°N. I think this is an overestimation, and I believe it could not be made any greater. Evidence from the deep-sea core (Fig. 1) suggests that the Spanish Sahara has always remained desert and, if anything, was more arid (had less cloud cover) during glacials. Extending these results to the globe, it therefore seems reasonable to assume that at 30°N the albedo is unchanged, but perhaps at 20°N it could be reduced slightly. The pole and the equatorial regions are assumed to have a constant albedo.

Fig. 4 The relationship between the surface-level temperature profile (*a*), the 4-km profile (*b*), and the infrared radiation emitted back to space at the 10° latitude intervals from the Pole to the Equator. (+, Present-day values, ⊙, the estimated glacial values—see text.)



With this first albedo assumption, the solar radiation curve is replotted (not shown), and then it has to be reduced by 1% to give a balance. The heat flux, across the latitudes, plots (not shown) well above the present-day value, except polewards of 65°N. I feel that the glacial heat flux should be greater than the present flux, but at all latitudes; clearly this first albedo is too high. The second albedo curve is deliberately drawn too low, making 65°N equivalent to 75°N. Balance is obtained with a 4% reduction, but the heat flux plots everywhere just below the present values. Some intermediate albedo is more realistic and I choose curve 3, which gives a balance for a 2.5% reduction; and the heat flux (the dashed curve in Fig. 3*b*) is everywhere greater than the present day. This glacial absorbed solar radiation curve is shown in Fig. 3*a*. If the albedo difference at 20°N is ignored, then there would be a 2% reduction. Assuming that present-day conditions are midway between a full interglacial and a full glacial, then I venture to suggest that the luminosity of the Sun has fluctuated with a 5% amplitude range and period of 10^5 yr.

It may be possible to reconstruct the temperature profiles for a glacial climate. Taking the 4-km profile as an example, the ten latitude points are moved along the empirical curve until they are at the appropriate glacial infrared radiation value, determined from the ground-level line. This gives a drop of 7 °C for the pole and for 80°N, 6 °C for 70°N, but a uniform fall of ~ 5.5 °C for the other points. In our present-day troposphere the average lapse rate of 5 or 6 °C km^{-1} is constant everywhere above 2 km, and does not depend on season. If the same lapse rate persists in a glacial climate, profiles at various heights may be constructed (Fig. 2*b*). A strong inversion between the surface and a height of 2 km appears polewards of 70°N as an annual feature.

It is interesting to compute, from the profiles, latitudinal thermal 'conductivity'. Physically, this would be a measure of the meridional component in the wind and ocean current structure. Using the surface-level profiles, the present-day and glacial conductivities are given in Fig. 3*b*. The high value in the tropics probably relates to the Hadley cell; but in the westerly wind belt, the meridionality of the transporting system is much reduced, and it seems that the glacial westerlies and ocean currents were noticeably more zonal than those of the present day.

It is interesting to note that the CLIMAP results do indicate a displacement of the Gulf Stream south of its present course, as if strong zonal winds directed it, during a glacial, more towards the Azores. Of course, the assumed increase in thermal gradient over the middle latitudes would encourage an eastward vector in the westerlies; and, treating the atmosphere as a heat engine, the whole circulation would have to be more vigorous. Evidence for this increase in vigour is already apparent in the deep-sea core work, some of which strongly suggests enhanced monsoonal activity[4,5]. Perhaps the Azores anticyclone intensifies, moves south-eastwards, and effectively blocks the Spanish Sahara from encroaching westerlies. A simultaneous intensification of the St Helena high would give an added 'push' to the monsoon.

I thank the Lamont–Doherty Geological Observatory for supplying the two cores V23-100 and V19-301 which have provided valuable data points.

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Received August 11, 1975; accepted January 13, 1976.

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Far infrared emission spectrum of the stratosphere from balloon altitudes

WE present here observations of the far infrared emission spectrum of the stratosphere taken with a balloon-borne interferometer from an altitude of 22 km above Churchill, Manitoba, Canada (latitude 58.7 °N, longitude 94 °W) in July, 1974. The instrument was part of a composite Atmospheric Environment Service of Canada gondola whose overall aim was the measurement, by several complementary methods, of the concentrations of minor constituents of the atmosphere and the monitoring of their changes with altitude and time during the flight. Many of the minor constituents of particular interest in the current debate on stratospheric pollution (O_3, H_2O, NO_2, N_2O, NO, HNO_3, HCl, SO_2) exhibit pure rotation spectra in the far infrared and are expected to contribute to the stratospheric emission spectrum. O_2, a well known and well behaved constituent of the stratosphere emits a series of weak magnetic dipole lines in this wavelength region, with intensities matching those of intrinsically stronger ones from less abundant species, and which can be used for spectral normalisation (see ref. 3).

Our preliminary data have been analysed by a method which uses synthetic spectra to allow for the weak O_3 lines and to effect a correct normalisation of the observations to the O_2 lines. The synthetic spectra were produced by a program described by McClatchey *et al.*[1], modified to use the instrumental convolution functions sinc *x* or sinc²*x* more applicable to Fourier transform spectroscopy (see ref. 2), with magnetic dipole transitions of O_2 added.

We used a simple rapid-scanning Michelson interferometer with no phase modulation, which produced interferograms with fringe frequencies for the highest wavenumbers of interest ($\sim 150\ \text{cm}^{-1}$) matching the maximum frequency response of the Ge(Ga) bolometer detector system[5] at Infra-Red Laboratories, Tucson, Arizona (~20 Hz).

The design of the system provided reproducibility between spectra in the wavenumber range 30–130 cm^{-1} to a resolution of 0.2 cm^{-1} at least equal to that of data taken from aircraft altitudes at equivalent resolution by phase modulation techniques[4–6]. Some of the more relevant details of the instrument and of the flight are discussed in the caption to Fig. 1, which shows a composite of two single spectra, displaced vertically to facilitate comparison.

Spectra were produced sufficiently rapidly that adequate height resolution during ascent (about 200 m per spectrum) and time resolution during sunrise and sunset (about 70 s per spectrum) were provided. Spectra could then be averaged if necessary after the flight to produce better signal-to-noise ratios. These spectra agree closely with those from aircraft altitudes[4–6], but with important differences. Strong H_2O lines which dominate most of this spectral region are considerably weaker at balloon altitudes. The O_2 lines, though still easily discernible up to 80 cm^{-1}, are somewhat weaker, particularly when compared with the comparatively strong Q branch emissions of O_3. This is as expected from the distributions of those constituents in the atmosphere. Weak O_3 lines pervade the whole region up to about 70 cm^{-1} and produce much of the small scale structure, making weak emissions from other constituents difficult to distinguish at this resolution. Strong H_2O lines still exhibit considerable emission in the wings of the lines, particularly those at 55, 56 and 88 cm^{-1}. No lines of H_2O other than

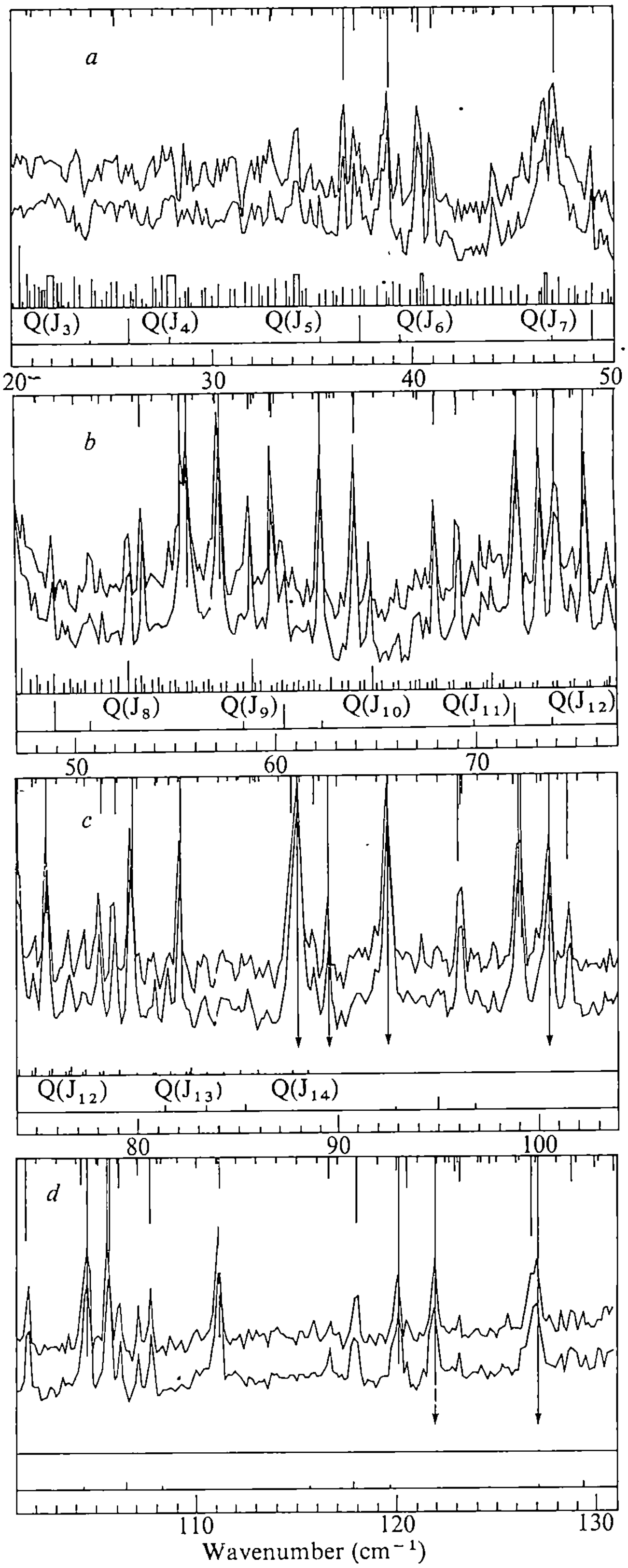

Fig. 1 Two emission spectra of the stratosphere between 20 and 130 cm^{-1} (resolution 0.2 cm^{-1}), taken at a zenith angle of 75°, from about 22 km in the anti-Sun azimuthal direction at 01.05 UT, July 23, 1974 (20.05 LT) *a*, 20–50 cm^{-1}, *b*, 50–75 cm^{-1}; *c*, 75–100 cm^{-1}; *d*, 100–130 cm^{-1}. H_2O line positions and relative strengths are shown above the spectra; O_3 lines and Q branches are delineated immediately below the spectra Magnetic dipole lines of O_2 are depicted below the O_3 line positions. The Ge(Ga) bolometer detector was fed by an *f*/1 5 TPX lens of 25 mm diameter, and was filtered by a mesh filter (at 2 K) a PTFE blocking filter (2 K), 0.4 mm black polyethylene (~100 K) and a TPX window at 253 K. The interferometer beamsplitter was a 12-μm thick Mylar (du Pont).

from the most abundant isotopic species are immediately obvious, nor is there evidence of emission from the dimer of H_2O, even under very careful analysis using synthetic spectra in regions of expected emissions. This finding is contrary to previous reports of aircraft measurements[7,8].

There is little evidence of emissions from other minor constituents in the preliminary analysis of these spectra. A peak is evident on an expanded plot at 37.8 cm^{-1} in the predicted position of a pseudo-Q branch of NO_2 (ref. 9). These emissions have been identified by Harries *et al.*[10] on spectra taken at aircraft altitudes, and a value for the integrated column density has been determined for that altitude. No peak of comparable magnitude to that observed in our spectra is predicted by the synthetic spectrum which includes O_2, H_2O and O_3 emissions. The line strength for this NO_2 emission is poorly known, however, and mixing ratios derived from this line are considerably above those obtained from other instruments on this flight. More definitive identification of the presence of emissions from this constituent at these altitudes must await spectra of higher resolution.

Of the expected HCl emission line positions only two are comparatively free of emission from other constituents, those at 41.6 cm^{-1} and 83.3 cm^{-1}. Expanded portions of the spectra at these wavenumbers indicate excess emission over that expected from weak O_3 lines as indicated by the synthetic spectrum, but it is not possible to say definitively whether emissions originate with HCl molecules. An upper limit to the mixing ratio of HCl on the basis of these data would be several parts per 10^9, considerably higher than the upper limit of 0.18 parts per 10^9, placed on this constituent in the lower stratosphere by Farmer[11] from near infrared observations.

Considerable structure is seen in the spectra between strong lines beyond about 80 cm^{-1} where weak O_3 lines should be negligible, and other molecular species are being examined as sources of these small but significant peaks.

The integrated column densities of H_2O and O_3 were obtained, and from several combinations of lines near to Q branches of O_3 the column densities of H_2O and O_3 above 22 km were determined as $1.1\ (\pm 0.6) \times 10^{18}$ and $5(\pm 2) \times 10^{18}$ molecules per cm^2, respectively. Further analysis of this kind, with careful fitting of data to synthetic spectra, particularly those calculated using multilayer atmospheric models, should yield more precise values of these constituents in the stratosphere. It is apparent, however, that values of line strengths for the magnetic dipole transitions of O_2 are needed to greater precision than are at present available (see ref. 12), particularly at the higher wavenumbers.

We thank Dr W. J. F. Evans, AES of Canada and the National Research Council of Canada for financial support. We also thank the staff of the Churchill Research Range and the balloon launch contractors, Raven Industries.

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Received November 17, 1975; accepted January 28, 1976.

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Lamb waves originating in non-geostrophic perturbations

A LAMB wave is a purely compressional wave mode in an isothermal atmosphere at rest above a horizontal rigid surface[1]. It travels only horizontally, with a phase velocity equal to the speed of sound and its energy density decreases exponentially with height. Bretherton[2] and Garret[3] have concluded that such a mode may exist in the temperature- and wind-stratified atmosphere of the Earth, with its energy mainly located at heights below 30 km. Lamb waves belong to the family of acoustic–gravity waves which, according to Rossby[4] and Obukhov[5], may be excited by non-geostrophic perturbations in a rotating stratified fluid until the fluid has returned to a quasi-geostrophic steady state. Here we report the observation of Lamb-wave generation in the troposphere during a geostrophic adjustment process.

On several days before and after the solar eclipse of June 30, 1973, simultaneous atmospheric and ionospheric observations were made at Tsumeb (19.2°S, 17.7°E) in southern Africa. On July 1, a microbarograph recorded unusually large ground-level pressure fluctuations, which, of course, were not caused by the eclipse. They have periods of ~ 1 h, and take place from morning until evening. Figure 1 shows a digitised form of the pressure record, but it is influenced by the filter characteristic of the microbarograph. Similar fluctuations superposed on the diurnal variations occurred in the Faraday rotation angle of the radio signal of a geostationary satellite and in the maximum electron density of the F2 layer (Fig. 1). The Faraday rotation is a measure of the ionospheric electron content which usually reacts very insensitively to perturbations in the electron gas. The maximum electron density is at a height of ~230 km and was deduced from vertical incidence ionograms which were not, however, available between 1210 and 1515 SAST. Around 1200 and from 1515–1800 SAST, it is particularly obvious that the electron density fluctuations show a strong correlation with the pressure fluctuations. A power spectrum analysis of all records shows two significant oscillations at periods of 90 and 60 min respectively, and each oscillation

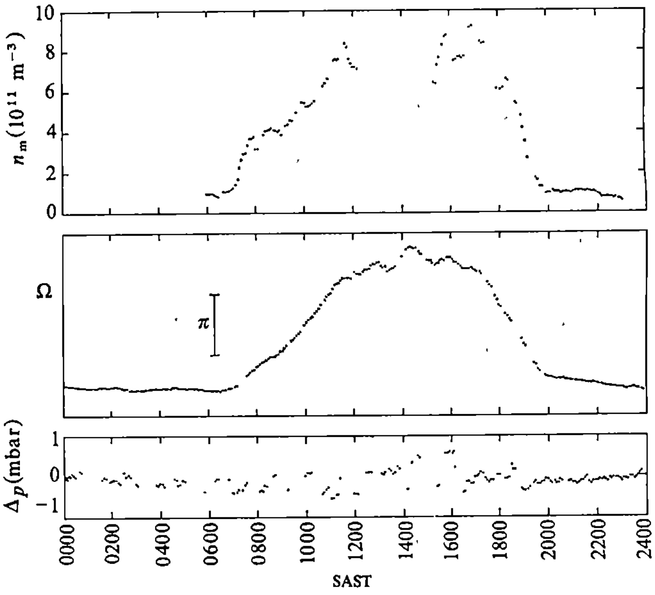

Fig. 1 Ground-level pressure variation Δp, Faraday rotation angle Ω of the 137-MHz radio signal of the geostationary satellite Intelsat IIF3, and maximum electron density n_m against South African Standard Time at Tsumeb on July 1, 1973.

has been isolated by a numerical bandpass filter. The two filters are characterised by centre periods of 90 and 60 min, and half-power widths of 38 and 18 min, respectively. Figure 2, for instance, shows the isolated 90-min oscillations confirming the strong correlation between the tropospheric and ionospheric events.

Additional Faraday rotation records were made at Outjo (20.1°S, 16.2°E) and Okahandja (22.0°S, 16 9°E), at distances ~250 km from Tsumeb. The filtered Faraday rotation data at all three stations show different phase angles, indicating that the oscillations are caused by propagating waves. For both periods, we find northward horizontal phase trace velocities of 220 m s^{-1}. The observed periods and velocities are typical of acoustic–gravity waves which propagate in the neutral gas causing perturbations of the ionised gas by ion–neutral collisions and by enhanced production and loss of ionisation[6,7].

The waves have been traced back towards the south by means of further Faraday rotation records and ionograms taken at several South African stations—additional microbarograms were not available. The 90-min wave could not be detected at Hermanus (34.4°S, 19.2°E), the 60-min wave not at Grahamstown (33 3°S, 26.5°E) Thus we may conclude that the waves were excited at low heights near these stations and could not reach the ionosphere above because their energy propagated on inclined ray trajectories[8]. Both stations are near the southern coast of Africa where, at the time in question, considerable non-geostrophic winds occurred (Fig. 3). Additional weather maps indicate that these winds were strongest during the daylight hours of July 1 The close spatial and temporal coincidence of the observed waves and ageostrophic winds thus confirms the theory that acoustic–gravity waves may originate in non-geostrophic perturbations.

It is reasonable to expect that the atmospheric response to a tropospheric source is dominated by Lamb waves because their energy is mainly located at low heights[9,10]. To test this hypothesis we shall compare our observations with calculated Lamb waves in a realistic atmospheric model. We take the 1972 Mean COSPAR International Reference Atmosphere which, below a height of 25 km, is completed by the 1966 US Standard Atmosphere Supplements. Winds are not taken into account since their meridional components, in particular, are unknown. The electron and ion number densities are described by α-Chapman layers with maximum densities of $4\times10^{11}\,m^{-3}$ at a height of 230 km. The wave-induced perturbations of the neutral and ionised gas are calculated from the linearised hydrodynamic equations including dissipative effects[11]. In Table 1, observed and theoretical results are compared where T=wave period, v=horizontal phase trace speed, r_n=relative perturbation of maximum electron density, r_p= relative perturbation of ground-level pressure, φ=phase difference between r_n and r_p. A calculation of the perturbation of the Faraday rotation angle is not possible for reasons given by Georges and Hooke[12].

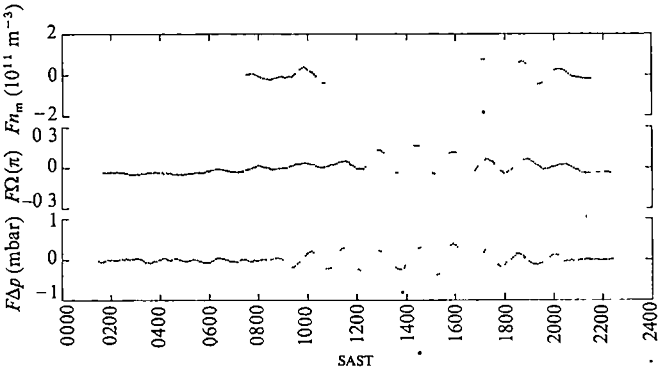

Fig. 2 Filtered records of ground-level pressure, Faraday rotation angle and maximum electron density.

The differences between observed and theoretical values of v most probably arise from the fact that, in deriving v from the Faraday rotation data, a horizontally-stratified ionosphere was assumed. A deviation of only 3° from the horizontal stratification, for instance, would introduce an error of some 100 m s^{-1} in the observed value of v because, at F-region heights, the Lamb waves behave like internal gravity waves with vertical wavelengths roughly five times

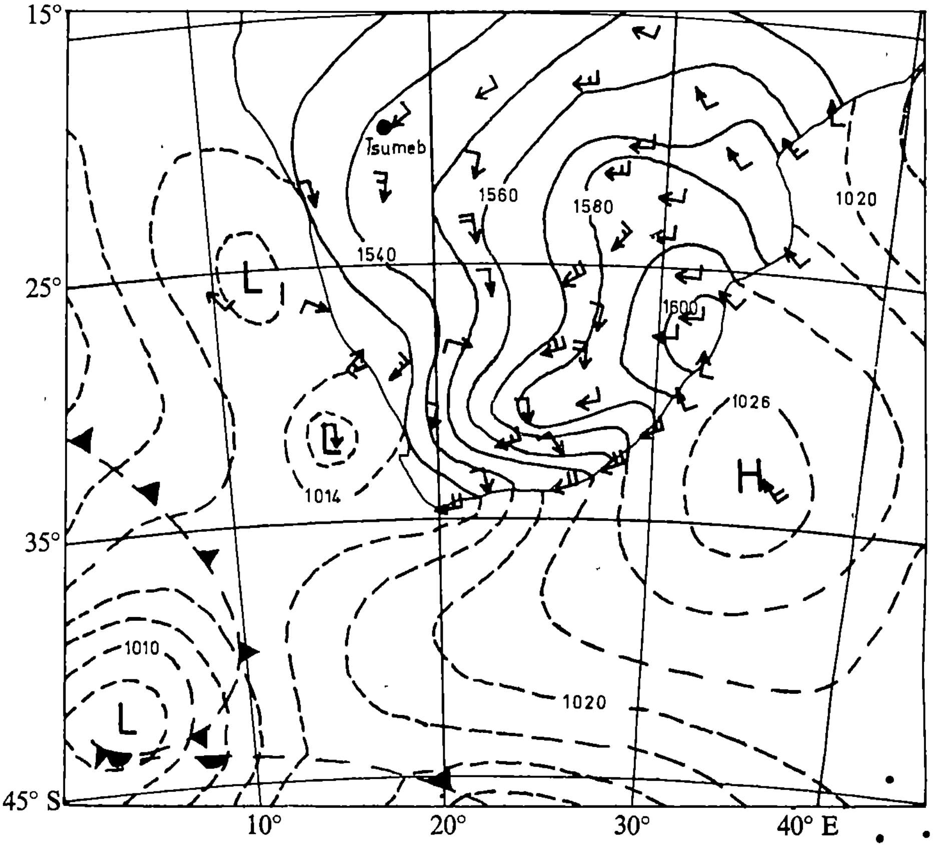

Fig. 3 Weather map on July 1, 1973, 1400 SAST. – – –, Isobars at MSL in mbar; —, contours at 850 mbar (gpm); ←, wind direction, each feather = 10 knots. (From Synoptic Weather Map, Weather Bureau, Republic of South Africa.)

Table 1 Comparison between observed and theoretical results

	$T = 90$ min Observed	$T = 90$ min Theoretical	$T = 60$ min Observed	$T = 60$ min Theoretical
v (m s^{-1})	220	310	220	310
$\|r_n/r_p\|$*	550	700	450	770
φ* (deg)	65	120	75	100

*Observed pressure perturbations are corrected according to the filter curve of the microbarograph. The values of φ have phase ambiguities of $2\pi n$.

smaller than the horizontal wavelengths[10]. The differences between the observed and theoretical values of $|r_n/r_p|$ and φ, on the other hand, possibly come from simplifications in the theory such as neglect of winds, surface friction and diffusion of the ionised constituents. In particular, the last two processes tend to reduce the differences in $|r_n/r_p|$. In spite of the highly magnified wave amplitudes at thermospheric heights, nonlinear effects play no role.

Other acoustic–gravity wave modes that can propagate in our atmospheric model give no better explanation for the observations than the Lamb mode. In particular, they yield values of $|r_n/r_p|$ which differ from the observed data by more than an order of magnitude. This is because other wave modes are ducted primarily in the upper atmosphere whereas the Lamb waves are guided by the Earth's surface[13].

I thank all who supported this investigation by providing observational data.

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Received November 3, 1975; accepted January 16, 1976.

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Ni and Co content of chondritic metal

WE have made a detailed study of the metal phases in some chondrites. Axon and Goldstein have reported[1] microprobe measurements of the Ni and Co contents at the interfaces between contiguous α and γ phases in seven metallic particles of high Co content from Apollo 14 and 15 lunar soils. The three particles of highest Co content had α phase in contact with clear homogeneous γ phases (Fig. 1), whereas the particles of lower Co content (Fig. 1) had Widmanstätten spindles of α phase in a matrix of high Ni γ phase in a manner analogous to that observed in certain Ni-rich ataxite irons. Axon and Goldstein have expressed the view that the Co contents of these particles are "higher than the meteorites of similar structure" and that they must have come from a different source.

In two instances, Olivenza (LL5) and Khanpur (LL5), we have encountered spindle-like exsolutions of the high-Co α phase in a matrix of high-Ni–high-Co γ phase. The α–γ interface values for the two meteorites are plotted together with measured bulk compositions in Fig. 1. It can be seen that the meteoritic metal is similar in structure and composition to the lunar soil particles, although the interface composition of the meteoritic γ phase shows less enrichment of Ni. In terms of the binary Fe–Ni equilibrium diagram, the LL5 metal seems to have γ-phase interface compositions appropriate to ~410 °C, in contrast to the value of ~350 °C observed for the lunar material.

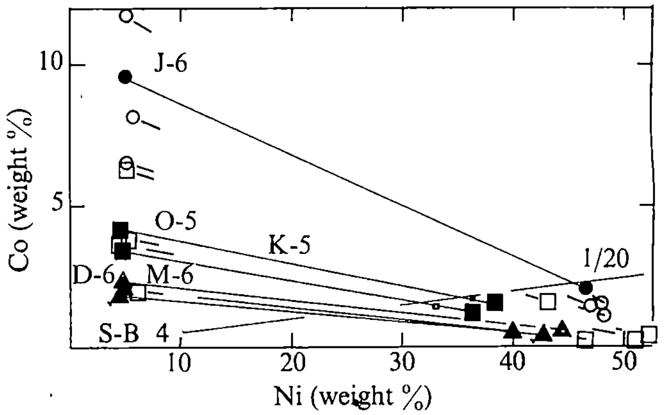
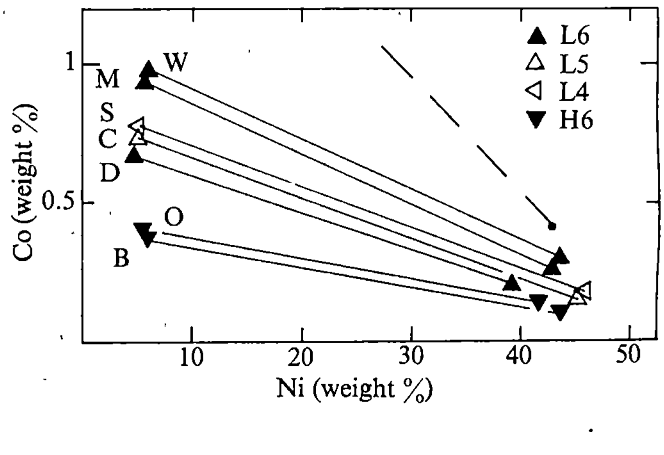


Fig. 1 Interface compositions for α and γ phases in the metal of LL chondrites (solid symbols) compared with high cobalt metal in lunar soils (open symbols). Circles, Clear γ phases; squares, ataxite type structures; triangles = zoned γ phases. J, Jelica; O, Olivenza; K, Khanpur; M. Mangwendi; D, Dhurmsala; S-B, Soko-Banja. Number, petrological grade of LL chondrites.

Figure 1 also includes α–γ interface values for Jelica (LL6). In this meteorite the α phase is extremely rare. When the α and γ phases are contiguous in Jelica, however, the γ phase is clear and compositionally unzoned, and in this case the bulk composition of the metal is almost coincident with the reported γ-phase composition. The bulk metal compositions for Khanpur, Olivenza and Jelica have a Co–Ni ratio of 1: 20 (Fig. 1). This represents the cosmic abundance ratio of the two elements. Therefore, the bulk Ni–Co contents of the metal phases in these three LL chondrites are consistent with a Prior's Law relationship in which iron partitions between the metal and silicate phases, whereas the more noble elements Ni and Co concentrate exclusively in the metal. The bulk composition of the metal is first established according to Prior's Law, and the distribution of Ni and Co between the α and γ phases is then controlled by tie-line relationships in the Fe–Ni–Co ternary equilibrium diagram. The lower part of Fig. 1 contains data for Mangwendi (LL6), Dhurmsala (LL6) and Soko-Banja (LL4). In each of these instances the α phase is contiguous with a compositionally zoned γ phase.

None of the samples in Fig. 1 shows visible signs of reheating in the metal phases. They are all observed falls, free from terrestrial corrosion and ablation heating. The silicate portions of these samples have been examined by Sears and Mills[2] who have given details of identification.

Fig. 2 Interface compositions between α and zoned γ phases in L and H chondrites. The broken line represents the lowest line of Fig. 1. W, Wold Cottage; M, Mauerkirchen; S, Saratov; C, Crumlin; D, Durala; O, Ogi; B, Butsura.



The same criteria were used to select an array of H and L chondrites from Sears' and Mills' samples. The results are shown in Fig. 2. The Ni content of the α phase is essentially the same in both Figs 1 and 2, but there is a distinct Co hiatus of about 1% between the LLα (Fig. 1) and the Lα phases (Fig. 2). There is also a marked change in the slope of the Ni–Co tie-lines between Figs 1 and 2, as may be seen from the broken line in Fig. 2, which represents part of the lowest tie-line (Soko-Banja) of Fig. 1.

No metallurgical reheating effects were observed in the samples shown in Fig. 2, but the kamacite (α phase) in both Wold Cottage (L6) and Mauerkirchen (L6) contained minor ε-shock transformation of the type that Axon and Boustead[3] have shown does not alter pre-existing compositions at α–γ interfaces. Within the limits of this investigation, no consistent differences emerged between the petrological grades L4, L5, L6. Any hiatus between the Co contents of α metal in H and L chondrites is, however, much less pronounced than that between L and LL chondrites. In contrast, the silicate compositions seem to show the reverse effect.

Reheating or shock-heating effects were identified in the metal from three of the samples from ref. 2 that are not plotted in Fig. 2. They are: Holbrook (L6), (α=5.8 Ni, 1.15 Co; γ=29.0 Ni, 0.45 Co); Gambat (L6), (2.86, 0.86; 16.2, 0.26), and Tennasilm (L4) (3.98, 0.75; 25.6, 0.25). Lines joining the α and γ compositions in these reheated samples of metal cross the assembly of Fig. 2 at a steep angle. It is noteworthy that the interface data for the metal in these reheated L chondrites are similar to those reported by Goldstein *et al.*[4] for two-phase α–γ or α–plessite structures in metal from Apollo 17 soils.

We thank Dr R. Hutchison (British Museum) and Dr A. A. Mills (University of Leicester) for supplying material. We can provide tabulations of the data on which Figs 1 and 2 are based.

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Received January 6; accepted January 19, 1976.

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Non-crystallinity and polymorphism in elemental solids

THIS paper shows a universal correlation of the non-crystalline state with polymorphism in elemental solids. This is demonstrated by a map constructed on a plot of the number of polymorphic forms (NPF) against the group number (GN) for all solid elements in the Periodic Table (Fig. 1). From this result, we propose that both the structure and occurrence of a non-crystalline solid are governed by the structures shown by crystalline states of the element.

The number of polymorphic forms was derived primarily from the data given by Eckerlin and Kandler[1], and by Pearson[2]. High pressure polymorphs were included because localised atomic packings in non-crystalline solids may assume the same bonding configurations as these exhibit. The elements with such high pressure polymorphs are As, Ba, Bi, Dy, Ga, Gd, Ge, Mn, rare earths, P, Pu, S, Sb, Si, Sn, Sr, Tb, Te and Yb. Polymorphs with a similar space group were counted just once, since the bonding natures may be almost identical among similar polymorphic structures, for example, B, Ce, Fe, Ga, Li, Mg, P, Pu, S and Te.

The GNs were arranged to give a progression in degree of bonding anisotropy from isotropic metallic bonding to anisotropic metallic bonding, to covalent bonding, to anisotropic semi-metals and back to isotropic metallic bonding.

Non-crystallinity is favoured in elements with a large number of polymorphic forms and a high degree of bonding anisotropy. Therefore the occurrence of non-crystallinity was described by regions with tie lines bounding the non-crystalline elements formed in similar conditions (see Fig. 1). We thus defined five regions distinguished by the most convenient, or least stringent, conditions for formation. Elements located on the boundary between two regions are best assigned to the lower numbered region.

In region I, non-crystalline solids can be prepared simply by quenching from the liquid state. This region contains the elements S, Se, P, As, and B.

In the upper part of region II, non-crystalline solids can be obtained by vapour, electrolytic or sputter-deposition near room temperature (elements such as C, Si and Ge). For the lower part of the region, Sb, Bi, Te, Fe and Ni require much lower deposition temperatures, ranging from liquid nitrogen to liquid helium temperature. Nickel and Fe were put in region II since they become amorphous in conditions similar to those for Te, Sb, and Bi.

For region III, non-crystalline solids can be formed only by vapour or sputter deposition at very low temperatures (near liquid helium temperature). Region III is defined by metallic elements Mn, Pu, Dy, La, Hf, V, W, Re, and Pd, and semi-metals Pb, Sn, Ge, and Tl. This region includes transition metals with GN from 4b to 8. Bonding characteristics for transition elements do not, however, change as much as the GN indicates, and therefore the occurrence of non-crystalline phases should be more dependent on the NPF than on the GN. Normally, the stability of the non-crystalline solids in region III is enhanced by impurities, thinness, and substrate conditions[3,4]. We therefore expect that a number of elements on the boundary between regions III and IV, such as Pb, Tl, Pu, Dy, La, Ce, Sm, Yb, U, Np and I to form non-crystalline phases under favourable conditions.

Non-crystalline solids are difficult to form for elements in region IV. Formation requires deposition at very low temperatures in the presence of stabilising factors including contamination and thin film effects, or favourable substrate surfaces. With the scant data available, region IV tentatively includes all group 2a and 3b elements at the left hand of the map. The elements Al, In, Zn, Cd, Hg, Cu, Ag and Au make up region 4 on the right hand of the map. Future work on elements in this region may require extra attention paid to the characterisation of the non-crystalline states as well as the control of experimental conditions, especially for the reported amorphous phases of Al, Be, Zn, Cd, Ag and Au.

There is no evidence for the occurrence of non-crystalline phases in Na, Cs, K and Rb, which make up region V. Because of the isotropic bonding characteristics and small number of polymorphs in group 1a, we believe these elements will not form non-crystalline solids.

This good correlation of polymorphism with the occurrence of elemental non-crystalline solids demonstrates that certain structural aspects of the crystalline state are relevant to the non-crystalline form. We therefore postulate that the structure of a non-crystalline solid is composed of a finite number of bonding states (or polymorphic bonding) with the same bonding characteristics (length, angle, and energy) exhibited by the polymorphs.

It is more reasonable to expect that crystalline state bonds exist in an amorphous solid rather than random packing arrangements. For each thermodynamically stable crystalline structure, there will be a limited number of bonding configurations constituting the lowest free energy state. The formation of non-crystalline phases, however,

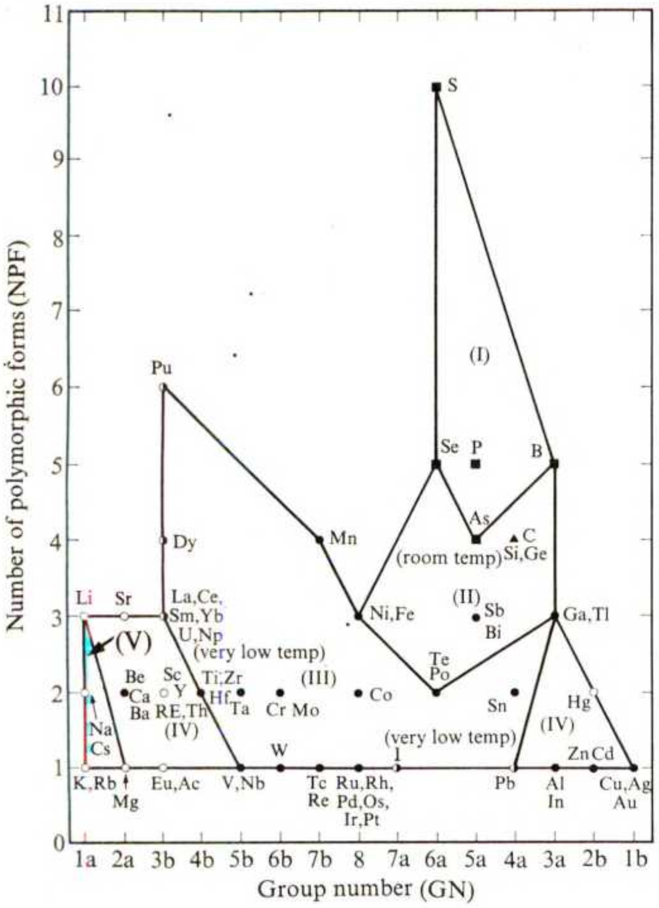

Fig. 1 Map showing the occurrence of elemental non-crystalline solids as correlated with the number of polymorphic forms (NPF) and group number (GN) of all the solid elements in the Periodic Table. The boundary lines are assigned to group elements conveniently into five regions representing the trend of formation conditions. The ease of formation of non-crystalline solids is illustrated by: ■, quenching from the liquid; ▲, formation by chemical or physical vapour deposition near room temperature; ●, formation by vapour deposition at low temperatures; half-filled circle, no data available, but we believe these can be formed in conditions similar to the ● data; and ○ no data available.

usually involves rapid quenching from high energy states (liquid or vapour) to prevent the forming of the lowest free-energy states. Instead, the atoms relax into a number of allowable bonding configurations separated by localised energy barriers. These allowable bonding types are those exhibited in the polymorphic structures. Therefore elements which have a large number of polymorphic forms will have a large number of available bonding states (energy minima) to accommodate the rapid relaxation process. The polymorphic bonding configurations would explain the low configurational entropy of non-crystalline phases as measured in Si and Ge[5]. The effect of GN on the occurrence of a non-crystalline phase can be understood in a similar context. Minimisation of the free energy by crystallisation becomes more unlikely as bonding anisotropy is increased from metallic bonds to covalent bonds, because of an increased 'jamming' probability.

Several recent observations support our concept of polymorphic bonding in non-crystalline solids. Structural models based on computer-simulated random packing give better agreement with diffraction results when variable bond lengths are allowed rather than single bond distances[6,7]. The optical properties of amorphous silicon are more consistent with the bondings in the high pressure tetragonal polytype than with the diamond structure[8]. Moreover, the fact that there can be more than one 'polytype' for an amorphous material, as in arsenic, in itself means that the non-crystalline state is characterised by specific bond types.

We are grateful to Dr R. D. Nelson for discussions. This work is supported by the Division of Physical Research, US Energy Research and Development Administration.

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Received August 12, 1975; accepted January 13, 1976.

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An early Ordovician vertebrate

HETEROSTRACAN fishes are the oldest known fossil vertebrates, and well preserved examples are frequently found in rocks of late Silurian and Devonian age, but in older rocks their remains are fragmentary and rare. Their oldest occurrence yet described is in the Harding Sandstone, Colorado (middle Ordovician[1,2]), where two genera occur; one of these has subsequently been recognised in marine limestones of similar age from Ontario, Canada[3].

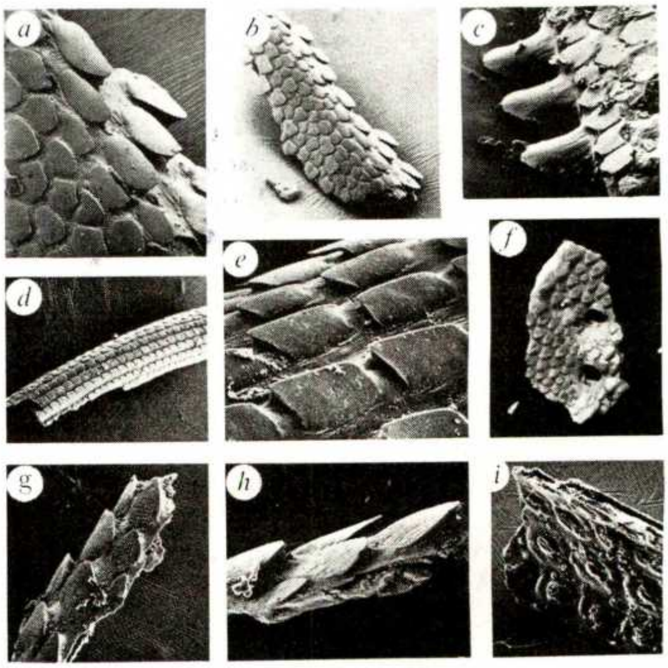

Fig. 1 Fragments of *Anatolepis heintzi* (*a*–*f*, *i*) and *Anatolepis* sp. (*g* and *h*) from the early Ordovician Valhallfonna Formation, northern Spitsbergen. *f* Was taken with a light microscope, the others with a scanning electron microscope. *a* and *b*, Holotype, PMO NF 3263/1 (× 54 and × 18, respectively), showing typical scale types with smooth external surfaces; *c*, PMO NF 3263/2, part of spine-like fragment (× 36), with transformed scales at edge; *d* and *e*, PMO NF 3263/3, elongate, U-shaped fragment with rhombohedral external scales (× 18), and detail (× 180); *f*, fragment with obliquely orientated, circular perforations, PMO NF 3263/4 (× 18); *g* and *h*, PMO NF 3263/5, fragment of second species of *Anatolepis* with grooved scales; *g*, from above (× 30); *h*, lateral view (× 94.5); *i*, inner surface of a fragment, PMO NF 3263/6 (× 54), showing low tubercles perforated at tip, and trilaminate structure at broken edge.

During the search for phosphatic microfossils from the early Ordovician Valhallfonna Formation, northern Ny Friesland, Spitsbergen[11], we recovered numerous small fossils which are identified as fragments of the earliest heterostracan, indicating that the earliest vertebrate occurrence predates that of the Harding Sandstone by about 20 Myr.

Whereas deposits containing later heterostracans were laid down in freshwater or brackish conditions[4], the earliest sediments are regarded as fully marine, indicating that the vertebrates had their origin in the same medium as other major metazoan groups[5,6]. The only recorded occurrence of vertebrate remains supposedly pre-Middle Ordovician are some small thelodont denticles—termed *Palaeodus* and *Archaeodus*—from the early Ordovician of the Russian platform[7]. These specimens are lost, and doubt has been cast on their authenticity (ref. 3 and S. Turner, personal communication). The Middle Cambrian problematicum *Eoichthys* Bryant[8,9] is regarded as unlikely to be vertebrate; indeed it was later[10] not considered as such by its original author.

The present finds were part of residues obtained by dissolving limestones in acetic acid, which otherwise include conodonts, chitinozoa[12], trilobites, brachiopods and radiolaria[13]. The associated fauna indicates a shallow shelf, fully marine environment, with a well oxygenated, soft, muddy sea floor, populated by a variety of benthic trilobites[14]. Vertebrate remains have been recovered from two horizons: 90–100 m from the base of the Olenidsletta Member and 30–50 m from the base of the Profilbekken Member. The former occurrence is with graptolites indicating an Arenig (early Ordovician zone of *Didymograptus hirundo*) age, the latter slightly younger, possibly earliest Llanvirn.

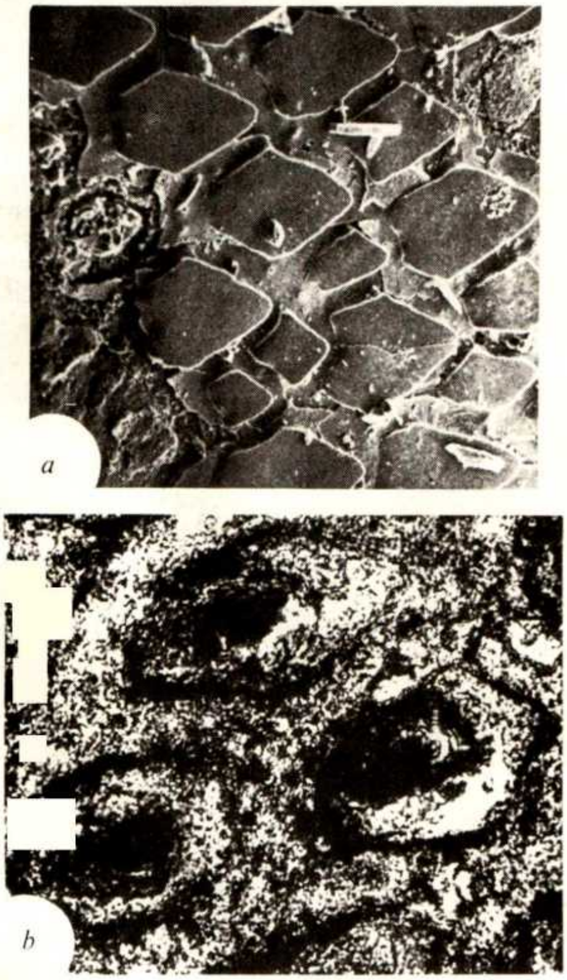

Fig. 2 *Anatolepis heintzi a*, PMO NF 3263/7 (× 300). Scanning electron microscope photograph of part of fragment showing circular structures beneath each scale; *b*, PMO NF 3264 (× 1,600), light microscope picture of similar fragment to *a*, photographed with transmitted light to show circular aspidin structures in middle of scales (NF 15, Fig. 10).

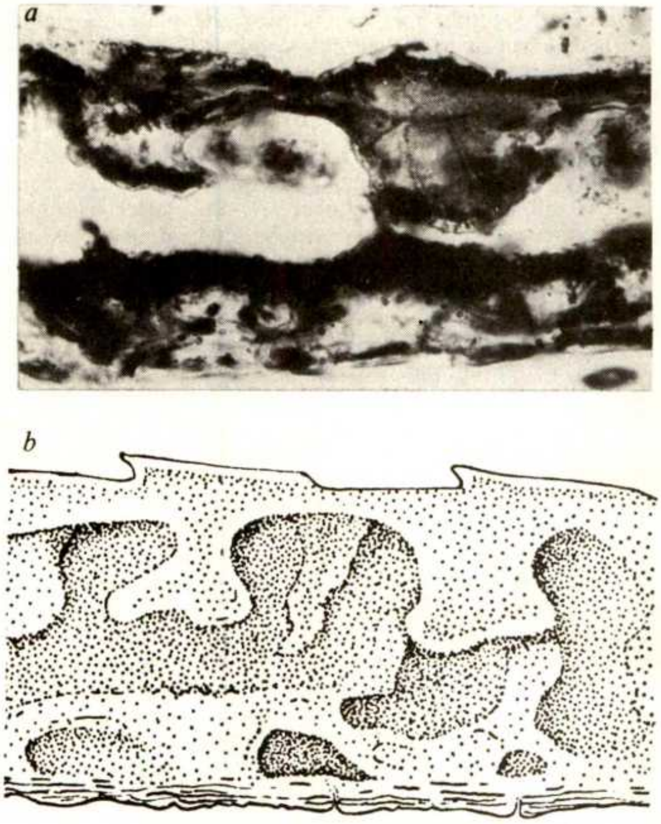

Fig. 3 *Anatolepis heintzi. a*, Photograph of thin section of PMO NF 3262 (×750), showing external scales, basal lamellar layer, intermediate cavernous aspidin layer. *b*, Generalised reconstruction of *Anatolepis* bone (×750).

The specimens range in length from 1 to 2 mm, and one or more edges are invariably frayed, suggesting that the fragments are parts of larger plates. Analysis by X-ray diffraction shows that fragments are composed of apatite but the possibility of the hydroxyl (OH) radical being present (in small amounts) cannot be eliminated. The outer surfaces of the plates are covered with minute, smooth, elliptical to rhomboidal scales 25–150 μm long; the larger scales are concentrated at the edges of the fragments, where they are frequently more or less imbricated. Plates are frequently 'doubled over' at the edges with larger scales (Fig. 1*a* and *b*), such fragments possibly being from the perimeter of the animal (and if this is so, indicating a dorsoventrally flattened overall shape). The inner surface of the plates carry tubercles of two sizes; the larger of these are perforated at their tips, these perforations presumably being the sites of dermal nerves or blood vessels (Fig. 1*i*).

Apart from flat or slightly curved fragments or 'edge' pieces, there are two particularly interesting fragments which, being covered with similar scales, are referable to the same animal. One is a spine (Fig. 1*c*) in which some of the scales are extended to form blades. The other fragment (Fig. 1*f*) includes small, circular openings, each equipped with a small lip externally and doubtless representing original openings in the exoskeleton. Since several groups of early agnathans had separate gill openings it is tempting to assume that these perforations represent the openings into the gill pouches, but they may also be pores into the lateral line system.

The plates are remarkably thin (70–100 μm) and delicate, and this explains why larger fragments have not been recovered. In section the structure of the plates is similar to that described for other heterostracans[15,16], with a basal lamellar layer, an outer, probably dentinous, layer carrying the rhomboidal scales, the two separated by a spongy 'aspidin' layer (Fig. 1*j*, Figs 2 and 3): the latter easily tears during sectioning. Both *Astraspis* and *Eriptychius* from the Harding Sandstone are much more robust and have completely different external tubercles—the latter variously lobate, frequently with broad, subparallel raised ridges, the former with hemispherical, radially grooved tubercles. External tubercles of both these genera have more than twice the average diameter of those of the new material from Spitsbergen. Because of these differences we propose a new name for the species described here—*Anatolepis heintzi* gen. et sp. nov. The type species is named after the late Professor Anatol Heintz, who contributed much to the study of early fishes. A distinctive second species, with minutely grooved external scales (Fig. 1*g* and *h*), has been recovered (one specimen only) from the lower horizon.

More material, preferably still *in situ* on the bedding surfaces, is needed before an attempt at a reconstruction of the whole animal can be made. The fragments alone are of interest because they prove that heterostracans were already present in the earliest part of the Ordovician (500 Myr ago) and show that they were then certainly Marine. They also indicate that the thicker armour of the middle Ordovician forms is likely to have been secondary[17]. Material at hand shows no evidence of cyclomorial growth[18] or fusion of smaller tesserae[19], and it is possible that skeletisation occurred all at once in *Anatolepis* rather than by fusion of individual tubercles. If such fusion occurred it may have been in an ancestor of Tremadocian or Upper Cambrian age. Our discovery may stimulate examination of acid residues of pre-Ordovician age for the remains of still earlier vertebrates.

We thank Drs A. C. Bishop, C. R. Hill and P. L. Forey for help in preparing this paper, and the Paleontologisk Museum, Oslo, for financial support.

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Received November 23; accepted December 29, 1975.

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Do nocturnal rodents run more efficiently at night?

An animal's activity is perhaps the most obvious physiological parameter influenced by circadian rhythms. Nocturnal animals move about and do their foraging during their hours of darkness. This higher level of activity is also synchronised with a higher basal metabolic rate as well as body temperature[1], and may also be reflected in metabolic needs for (the increased) locomotion occurring during the active period. Since the animal is using more energy simply to maintain its basic needs, there may also be a higher energy requirement for moving about. We have examined this, and have found that the increased basal rate persists as the constant difference (at different speeds), between the day and night performances of gerbils.

Three male gerbils (*Meriones unguiculatus*, weight 62–85 g; average 71.7 g) were purchased from local pet stores, and fed rat food and water *ad libitum* supplemented with seeds, fruits and vegetables. Between 20 and 26 h before each run their food was removed. The gerbils were placed on a 12-h light (0600–1800) and 12-h dark (1800–0600) cycle two weeks before the experiment. The animals were run under ordinary room light for the light runs and under dim red lights for the dark runs (the lighting conditions seemed to have no effect on their running ability). Running took place on a treadmill 40 cm long and 10.5 cm wide enclosed in a Lucite box (13×15×50 cm) kept at 20–22 °C, and the gerbil's speed was measured by timing revolutions with a stop-watch; it could be maintained to within ±5%. Oxygen consumption was measured on a Beckman model F-3 oxygen analyser calibrated to within ±0.005% O_2 (1% of full scale deflection) by lowering the pressure within the measurement cell. Room air was pulled into the chamber at a rate of 226 l h^{-1} (STP) ±3% giving a 75% turnover of the air in the chamber in 3.6 min (assuming complete mixing). The chamber was kept at a slight negative pressure, ensuring that all the air exhaled by the animals would be drawn into the oxygen analyser. After the gerbils had run for at least 20 min at each speed and were running well, data were taken for each 10-min period when the deflection of the oxygen analyser trace showed an oxygen variation of no more than ±0.005%. Oxygen consumption was calculated by multiplying the air flow by the change in oxygen concentration. This value was then multiplied by 1.0425, on the assumption of a respiratory quotient (*R*) of 0.84, which would give a maximum ±4.25% error for *R* values of from 0.70–1.0 (ref. 6). In fact, the *R* value probably did not vary appreciably, since

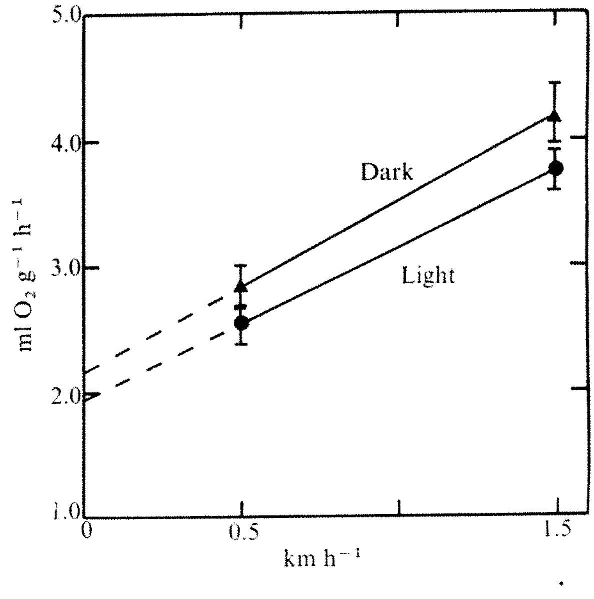

Fig. 1 Oxygen consumption of gerbils running during their dark (▲) and light periods (●) at 0.5 and 1.5 km h^{-1}. Vertical bars with horizontal caps indicate ±2 s.e.m.; there was no overlap of the means ±2 s.e.m. The lines joining the means are extrapolated to the *y* intercept. The slope for the dark period running is 1.35 ml O_2 g^{-1} km^{-1} (s.e.m. =0.140); for the light period it is 1.21 ml O_2 g^{-1} km^{-1} (s.e.m. =0.107).

the animals were all in the same nutritional state during their runs.

For both running speeds used here (0.5 and 1.5 km h^{-1}) the oxygen consumption of animals running during their dark period was significantly higher than when they ran during their light period (Fig. 1). It takes more energy to run a given speed during the dark (active) period. The increments in oxygen consumption for the 1 km h^{-1} difference in speed between 0.5 and 1.5 km h^{-1} were, however, about the same, and were below those predicted for quadrepedal mammal of that size. The increment was 1.35 ml $O_2\ g^{-1}\ h^{-1}$ in the dark period, and 1.21 ml $O_2\ g^{-1}\ h^{-1}$ in the light period, compared with predicted value of 1.52 ml $O_2\ g^{-1}\ h^{-1}$ for a 72-g mammal[2]. The *y* intercepts of the lines (2.17 and 1.94 ml $O_2\ g^{-1}\ h^{-1}$) are in good agreement with the predicted value of 2.04 ml $O_2\ g^{-1}\ h^{-1}$ (ref. 2).

The oxygen consumption data may be interpreted as if the resting requirement is higher during the dark period but the metabolic increase needed for running is the same during either period, in agreement with measurements of activity and metabolism in rats during dark and light periods[3]. Lizards and mammals of the same weight also show the same metabolic increments for running, even though the lizard has a much lower basal oxygen usage[4].

Splitting the metabolic levels shown in Fig. 1 into a resting and a running component shows that the increment from running will be about the same whether the running occurs during the light or the dark period. Then, since the time of the day the animal chooses to run a given distance will affect neither the metabolic increment due to running nor the total resting oxygen consumption, the total oxygen consumption over a 24-h period will be the same. Whatever other advantages may accrue to the gerbils as nocturnal animals, such as safety from predators or decreased evaporative water loss during the cooler night periods[5], there is no metabolic adaptation for more efficient running when the animals run the most.

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Increased incidence of triploidy in embryos derived from mouse eggs fertilised *in vitro*

FERTILISATION *in vitro* has now been achieved in several mammalian species, including rabbit[1], mouse[2], golden hamster[3], rat[4], and man[5]. The possibility exists that the technique may increase the incidence of chromosomal anomalies through any of several mechanisms including meiotic disturbance in the oocyte associated with superovulation, fertilisation by two or more sperm as a result of a high sperm concentration and/or a change in the natural block to polyspermy, altered conditions of competition between sperm with normal and abnormal genomes, and disjunctional irregularities during early cleavage of the fertilised egg. To determine whether any of these possible types of abnormality are of real significance will require extensive investigation. As yet the limited published data from human[6,7] and rabbit[8] embryos are either too few to be useful or lack control observations. We have therefore made a cytogenetic study of the incidence of triploidy in mouse embryos following fertilisation *in vivo* and *in vitro* and have found a highly significant increase in triploidy in the *in vitro* group.

Care was taken in both series to arrange that the eggs fertilised were 'fresh' (that is, non-aged). In both groups, eggs were from 2–3 months old (C57BL/10×CBA) F_1 females with sperm provided by outbred TO males. All of the females were induced to superovulate with 5 IU pregnant mare's serum (PMS) (Gestyl, Organon), followed approximately 48 h later by 5 IU human chorionic gonadotropin (HCG) (Pregnyl, Organon). For fertilisation *in vivo* HCG was administered 12–13 h before the estimated time of natural ovulation. Females were paired with males and examined for vaginal plugs the following morning; the day of finding a plug was taken as day 1. For fertilisation *in vitro*, females were killed 13 h after HCG administration and their unfertilised eggs were immediately mixed with sperm. Thus, in both groups the eggs were the same approximate age at the time of association with sperm.

Table 1 Chromosomal analysis of mouse embryos derived from superovulated eggs fertilised *in vivo* and *in vitro*

	40	±40	±60	Triploidy (%)
In vivo days 3 and 4	162	17	5	2.7
In vitro 72-h culture	124	26	22	12.8

Experiments using the same *in vitro* system have indicated that sperm–egg union is complete within 1.5 h of gamete mixing (unpublished), a time which correlates well with *in vivo* studies indicating that sperm penetration can be detected within 1 h of mating[9,10].

Whittingham's medium based on Tyrode's solution[11] with 0.5 mM pyruvate was used for fertilisation *in vitro*. The medium for fertilisation contained 32 mg ml^{-1} bovine serum albumin (BSA, Armour); that for culture contained 4 mg ml^{-1} BSA. The preparation of the medium and dishes for experimental use followed the methods of Hoppe and Pitts[12]. A sperm suspension was prepared by gently forcing the contents of two epididymides into the medium under paraffin oil with watchmakers forceps. After 20 min allowed for dispersal, a 1 : 9 dilution of the suspension giving a final concentration of 1.5×10^6–3.0×10^6 ml^{-1} was used for fertilisation.

Eggs were released directly into the sperm and incubated at 37 °C for 3–6 h, then washed and cultured in droplets of medium under oil. After 72 h, embryos were at the 8–16-cell stage, and at that time the medium in the droplet was changed to one containing 10^{-8} M vinblastine sulphate (Velbe, Lilly) prepared in culture medium. Following incubation for a further minimum period of 3 h, chromosome preparations were obtained from the embryos.

In the experiments the mean *in vitro* fertilisation rate was 77.7% (two-cell/total eggs), and 89.6% of these two-cell embryos reached the 8–16-cell stage used for chromosomal examination. If cultured further, rather than being fixed, 80–90% of embryos would have been expected to form blastocysts, according to previous experience. Further, offspring of both sexes have been obtained after embryo transfer[13].

Naturally mated females were injected on days 3 or 4 with 0.1 ml Velbe (1 mg ml^{-1}), and killed 2–5 h later to allow the recovery of embryos. The embryos of both *in vivo* and *in vitro* groups were placed into 1% sodium citrate for 8 min, transferred to acid-cleaned slides, fixed rapidly with 3 drops 3 : 1 methanol–acetic acid and air dried (ref. 14, and E. P. Evans, personal communication). The preparations were stained with toluidine blue for cytogenetic analysis. Analysable preparations were obtained from 33% of the 528 embryos in the *in vitro* series and from 44% of the 414 embryos in the *in vivo* series.

The chromosomes were counted in all suitable blastomeres at mitotic metaphase (Table 1). The majority of counts were confidently either 40 or 60. No attempt was made to sex the embryos by identification of the Y chromosome. Some of the counts shown in Table 1 as ±40 may have been genuine aneuploids but no instances of $2n/3n$ chimaerism or levels of polyploidy higher than $3n$ were identified. The difference between the frequencies of triploidy recorded in the *in vivo* series (2.7%) and the *in vitro* series (12.8%) is highly significant ($\chi^2=11.47$, $P < 0.001$).

The mean frequency of triploidy among 3.5-d embryos derived from naturally ovulated eggs of 6 non-silver mouse strains was 0.9%, according to data given by Beatty[15]. The mean frequency in the exceptional silver strain was considerably greater, reaching 9.5% in one series. In an investigation of 1,359 preimplantation embryos from naturally ovulated (C3H×101)F_1 females, 13 (1.0%) were triploid (E. P. Evans, personal communication). The latter embryos were obtained following morning matings and the eggs are therefore likely to have aged for several hours before fertilisation. Estimates of the frequency of triploidy among the preimplantation embryos of naturally mated, superovulated mice are 0.3% (ref. 16), 1.2% (ref. 17) and 1.8% (P. A. Neighbour, personal communication), in addition to our own figure of 2.7%. We have been unable to find an estimate of the contribution of superovulation itself to the incidence of triploidy in the mouse, which would require comparison with data from the embryos of matched, naturally ovulated and fertilised females.

Estimates ranging from 1.2% to 8.5% polyspermy in superovulated eggs fertilised *in vitro* have been derived from data obtained using Swiss mice[18,19]. No control data from natural matings were provided so it is not possible to say anything about the significance of these figures. High frequencies of polyspermy have been recorded in superovulated eggs of the golden hamster fertilised *in vitro*, for example by Barros *et al.*[20], but such eggs have not been cultured past the two-cell stage[21]. All of these estimates were based on the number of pronuclei or sperm heads within the vitellus. Such observations provide no assurance that a synkaryon will result or that the egg will cleave. They cannot, therefore, have the same validity as counts of chromosomes at mitosis for the estimation of the proportion of resulting morulae that are triploid.

Counts of chromosomes have been reported in 26 human blastocysts obtained following *in vitro* fertilisation[6,7]. All were said to have been diploid or approximately diploid. In view of the small number studied, these data are of limited value in excluding abnormalities of ploidy.

The explanation we favour for the striking rise in the incidence of triploid embryos following fertilisation *in vitro* is dispermy. This could be related either to a much higher sperm concentration *in vitro* than *in vivo*[22] or to an alteration of the mechanism that normally prevents polyspermy. Disturbance at the second maturation division, leading to an increased frequency of digyny is also possible. The frequency of digynic triploidy, however, is unlikely to be great since the proportion of eggs with second polar bodies at the time of transfer to the culture medium is closely correlated with the proportion later observed to cleave (unpublished). A marker chromosome experiment to discriminate between these possibilities is in progress.

We thank Drs C. E. Ford and R. D. Barnes for discussions and suggestions. G.R.P., a visiting worker at the Clinical Research Centre, was a member of the MRC External Scientific Staff during the course of this investigation.

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Received July 7, 1975; accepted January 28, 1976.

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Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man

THE natural ecology of *Escherichia coli* and its infectious plasmids is not understood, although there is suggestive evidence that animals may serve as reservoirs for *E. coli* found in humans[1,2]. Investigation in this area becomes additionally important in view of the practice of introducing plasmids with pieces of foreign DNA into *E. coli*[3,4]. During an examination of the effects of antibiotic-supplemented animal feed on flora of farm animals and human personnel (ref. 5 and S.B.L., G.B.F. and A.B.M., unpublished), a study was initiated to determine if *R* plasmids and their *E. coli* hosts were naturally transferred among chickens and from chickens to human handlers. Our results illustrate the spread of antibiotic-resistant organisms from chicken to chicken and from chicken to man.

We used a temperature-sensitive chloramphenicol-resistance gene as a marker to identify a particular plasmid. *Escherichia coli* D1-7 (ref. 6), carrying *R222*, was treated with nitrosoguanidine[7] and a mutant expressing temperature sensitivity to chloramphenicol was selected from the surviving bacteria. The temperature-sensitive chloramphenicol-resistance property was easily transferred to other hosts. This finding indicated its presence on a plasmid. This mutant plasmid, designated *pSL222–6*, expressed resistance to chloramphenicol, tetracycline, sulphonamides and streptomycin at 32 °C, but at 42 °C did not express chloramphenicol resistance. *pSL222–6* was transferred by conjugation into two morphologically distinct *E. coli* isolated from chickens. An inoculum containing a mixture of the two recombinant bacteria was introduced directly into the intestines of four chickens by means of a tube placed in the cloaca. The chickens were caged separately and fed tetracycline-supplemented feed (Pfizer; tet-feed) to enhance colonisation with this tetracycline-resistant *E. coli*.

A repeat inoculation was carried out 4 d later although subsequent analysis showed that already at this time all

Table 1 Number of chickens excreting *E. coli* containing *pSL222–6**

Day	Cage A	Cage B	Cage C	Cage D
0	0	0	0	0
7	1	4	0	0
14	1	6	0	0
49	5	8	0	0
56	6	8	0	0
63	7	12	0	0
% Chickens excreting mutant *R* plasmid	14	24	0	0

*Does not include inoculated chickens placed in cages A and C; chickens in cages A and B were fed tet-feed; C and D received normal feed.

of the faecal *E. coli* isolated from these chickens were resistant to chloramphenicol at 32 but not at 42 °C. Two chickens were then placed in two cages each containing 50 chickens. One cage (A) was placed on the tetracycline-supplemented feed; the other (cage C) was not. Two other cages containing chickens with (B) and without tet-feed (D) were also maintained in the barn at about 50 feet from the experimental cages. In this way, the effect of tet-feed on the spread of the marked plasmid from inoculated chickens to other chickens could be evaluated.

Samples of faeces were taken weekly using a sterile swab (Handi-Swab, Fisher) from the cloaca and cultured on MacConkey agar plates at 30 °C. These master plates were then replica plated on to MacConkey agar containing chloramphenicol and incubated at 30 and 42 °C. Table 1 illustrates the spread of the mutant plasmid from the initial two chickens to the rest of the animals in these cages. After 2 months, *E. coli* with temperature-sensitive chloramphenicol resistance were detected in 14% of chickens in cage A. Of particular note was the finding of *E. coli* with this particular *R* plasmid in faeces of 24% of chickens in a distant cage (B). None of the bacterial samples from chickens in cages not placed on tet-feed (C and D) demonstrated the mutant *R* plasmid.

Since only the plasmid was marked in this first experiment, a second study was carried out to determine whether the plasmid was being spread by way of the host bacterium. Plasmid *pSL222–6* was introduced into a mutant strain of chicken *E. coli* resistant to nalidixic acid (Nal^R). (*Nal* resistance is not normally found among *E. coli* in chicken gut flora.) The Nal^R *E. coli*-bearing *pSL222–6* were introduced by cloacal inoculation into four chickens previously fed tet-feed. These chickens were placed in a cage of 18 chickens also eating tet-feed and excreting organisms resistant to tetracycline, and the presence of the doubly marked *E. coli* followed (Table 2). After 1 week, 7 of the 18 chickens were excreting Nal^R *E. coli*, all of which showed temperature-sensitive chloramphenicol resistance. By 5 weeks these *E. coli* were detected in all the chickens in the cage. Spread was more rapid than in the previously described experiment. About 20–30% of *E. coli* in the flora of these animals was Nal^R (*pSL222–6*).

Over a two-month period faeces from eleven family members living on the farm and three laboratory workers responsible for the sampling of the chickens were tested for the presence of *pSL222–6*. On two occasions the plasmid was detected in human faeces. The first occurred in the faecal sample from the 12-yr-old son of the farm owner; 2 d after he had cleaned the cage in which chickens harbouring *E. coli* with this plasmid were housed, 78% of the coliforms in his faecal flora were resistant to chloramphenicol and this resistance was temperature sensitive. The *E. coli* also showed resistance to streptomycin and sulphonamides, but not to tetracycline. These resistances were not transferable. A similar non-transferable tetracycline-sensitive segregant of *pSL222–6* was found in *E. coli* from chickens in the cleaned cage. The presence of a plasmid in this *E. coli* was verified by the identification of a fast-moving peak of labelled DNA in an alkaline sucrose gradient (data not shown). Furthermore, other plasmids of the same incompatibility group (FII) were unstable when conjugated into this *E. coli*. This finding indicated the presence of a plasmid of the same incompatibility type as *pSL222–6*. Since typing of *E. coli* was not possible and the *E. coli* were not marked we could not determine whether the organisms were of human or chicken origin.

The second human host observed excreting *E. coli* with *pSL222–6* was an animal handler. One week after she had sampled chickens inoculated with the Nal^R *E. coli*-bearing *pSL222–6*, her faecal flora showed the presence of temperature-sensitive chloramphenicol-resistant *E. coli*. These *E. coli* were also resistant to nalidixic acid. All three other resistances on *pSL222–6* were also present. In conjugation the resistances in this *E. coli* were transferred together.

Subsequent faecal specimens from these two individuals 3 and 5 d after the initial sample did not reveal the presence of these particular organisms. Neither individual was taking antibiotics. Since only faecal swabs were examined, further studies would be necessary to determine whether these organisms were present to small extents in their intestinal tracts.

Our results demonstrate the spread of resistant organisms from chicken to chicken and from chicken to man. In studies of 1-d-old chicks, Smith[8] found *E. coli* from one group of chickens in faecal samples from a second group of chickens within 2 d of their being mixed in a cage. In the present study spread of the mutant *R* plasmid and Nal^R *E. coli* was detected in adult chickens in the same and distant cages. The mechanism of spread, whether by air or on the clothes of the feed handler, is not known. Cage-to-cage spread of bacteria is probably a common occurrence in a farm environment, followed by subsequent spread of plasmids and bacteria among the chickens in the cage.

In the light of our findings, transfer may also occur among other animals and between other animals and man. In the absence of direct contact and of selective pressure, such as that exerted by antibiotics, the frequency of spread is probably small. The subsequent transfer of animal *R* plasmids to human *E. coli* in the intestine, however, could occur[9] especially if the recipients were taking an antibiotic related to resistance determined by an *R* plasmid[10]. Although as few as 10^4 organisms can produce colonisation of the human intestinal tract[11] most studies *in vivo* have indicated that more than 10^6 bacteria need to be ingested[12,13]. This seems too many to have been ingested without their

Table 2 Spread of newly introduced R^+ *E. coli* among chickens

	Week 0	Week 1	Week 3	Week 4	Week 5
Number of new chickens excreting Nal^R *E. coli* (*pSL222–6*)	4	7	3	2	2
Accumulated number of chickens excreting Nal^R *E. coli* (*pSL222–6*)	4	11	14	16	18

Four chickens excreting Nal^R *E. coli* bearing temperature-sensitive chloramphenicol-resistant *R* plasmid *pSL222–6* were introduced into a cage of 18 chickens previously fed tetracycline and excreting tetracycline-resistant *E. coli*. Spread of Nal^R *E. coli* was followed by replica plating initial faecal samples, from MacConkey agar plates, on to (1) MacConkey agar + chloramphenicol at 30 and 42 °C; (2) MacConkey agar + nalidixic acid (30 μg ml^{-1}) at 37 °C. All Nal^R colonies were temperature sensitive for chloramphenicol and vice versa.

knowledge by the two individuals in this study. The results suggest a different route of entry or a different culture medium for the ingested bacteria, for example, by way of the nasal passages to sputum to the gastrointestinal tract.

These data bear on the widespread use of antibiotics in veterinary and medical practice and on the proposed use of *E. coli* as a vehicle in genetic engineering experiments. Further studies on the conditions affecting transfer and mixing between animal and human bacteria will be important in evaluating the consequence of antibiotic-resistant bacteria in animals in contact with man.

This study was supported by the Animal Health Institute, Washington, DC, and a Research Career Development Award to S.B.L.

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Thermostability studies for investigating non-electrophoretic polymorphic alleles in *Drosophila melanogaster*

ON theoretical grounds it is generally believed that most structural genes have common isoelectrophoretic alleles[1–3]—polymorphic alleles that cannot be discovered by electrophoresis—but there is no direct evidence for this. Methods that might be used to search for such structural variability (involving, for example, thermostability or kinetic studies) have not often been adopted as screening procedures though they have been used to compare already identified electrophoretic alleles. This is not only because such methods are often unsuitable for screening purposes. Even when a screening method can be applied, its efficiency in revealing existing structural variability is not known *a priori*, not even approximately, as for electrophoresis. Moreover, this unknown efficiency is presumably very low because although electrophoresis, being a separation technique, separates the products of two alleles in heterozygotes, other screening procedures only pick up the combined products which may or may not show an intermediate characteristic. It would usually be extremely difficult to distinguish between the three types. Thus, for any enzyme for which such a method is available, a two-step procedure seems convenient: (*a*) to test it on already known structural (electrophoretic) alleles of that enzyme, and if it turns out that it is efficient (*b*) to use the method in a search for structural isoelectrophoretic alleles. It must be assumed that these two types of structural differences are, on average, equally likely to affect the parameter being studied.

Drosophila melanogaster provides convenient material for such an approach because it is easy to synthesise isogenic lines for whatever gene has been mapped. In this way all relevant comparisons can be made between homozygotes for the alleles being studied. Phosphoglucomutase (PGM) depends in *D. melanogaster* on a single autosomal gene (3: 43.6) (ref. 4) with two common, Pgm^{A} and Pgm^{B}, and several rare electrophoretic alleles, Pgm^{C}, Pgm^{D}, Pgm^{E} and Pgm^{F} (ref. 5). Therefore this gene is quite suitable for this purpose. Thermostability is relatively simple to study, even for homogenates of a single fly. Two procedures were used to evaluate the thermostability of the various allelic PGMs studied: (1) incubation of two aliquots of a homogenate of several *Pgm* isogenic flies at two temperatures followed by electrophoretic analysis and (2) incubation at two temperatures of two slices of a starch gel after electrophoresis of an homogenate of a single fly followed by the addition of the PGM-staining mixture[6]. Temperature and time of incubation were decided empirically.

Figure 1 shows that our technique enabled us to classify any PGM band as heat resistant (R) or heat sensitive (S) on an alternative basis. Moreover, since heterozygous individuals were found in which the product of one *Pgm* allele was resistant and the product of the other *Pgm* allele was sensitive, we conclude that this property depends on the *Pgm* structural gene.

Presumably, there were available for heat-sensitivity studies several possible rare *Pgm* alleles because, besides those variants which are electrophoretically different, variants with the same electrophoretic behaviour but different in origin—if they are rare—are likely to be structurally different. Table 1 shows a large heterogeneity as regards heat sensitivity between both electrophoretically different *Pgm* alleles and rare alleles with the same electrophoretic behaviour but different origins. Moreover, as it might be expected, the latter heterogeneity was not found within the same population. It therefore seems reasonable to compute as certainly or probably distinct alleles any two *Pgm* rare variant alleles differing from each other in electrophoretic behaviour or origin, respectively. Of 16 such variant alleles 10 turned out to be R (5 Pgm^{C} and 5 Pgm^{D}) and 6 S (2 Pgm^{C}, 3 Pgm^{E} and 1 Pgm^{F}). Conclusive

Table 1 Classification of several rare *Pgm* variant alleles based both on their electrophoretic and heat-sensitivity behaviour

Origin of the strains			PGM phenotypes							
		Electrophoretic	C		D		E		F	
		Heat sensitivity	R	S	R	S	R	S	R	S
Laboratory			1	—	2	—	—	1	—	—
Puglia	Castellaneta		2	—	1	—	—	—	—	1
	Otranto		2	—	—	—	—	—	—	—
	Corato		1	—	—	—	—	—	—	—
Sicilia	Ranna		—	1	6	—	—	1	—	—
	Archi		1	—	4	—	—	—	—	—
	Vittoria		—	1	1	—	—	2	—	—

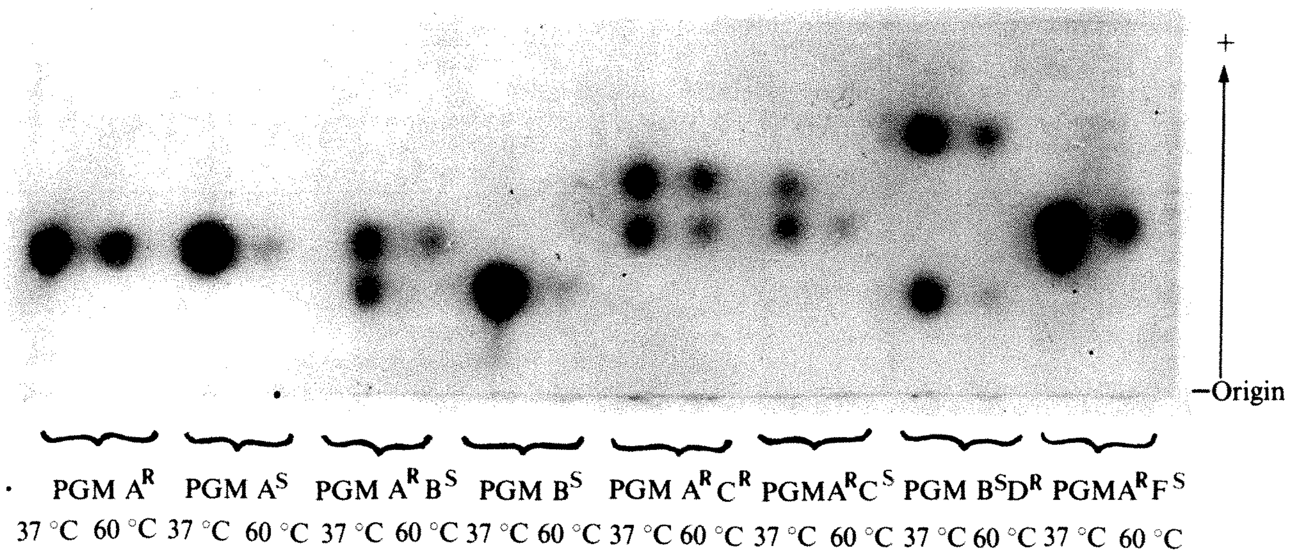

Fig. 1 Heat-sensitivity behaviour of various *Pgm* alleles. In the first procedure, for each strain to be examined 50 adult flies were homogenised in 1 ml of Tris-maleate buffer, *p*H 7.4, 10^{-3} M, diluted with 4 ml of the same buffer, centrifuged at 3,000*g* for 15 min and the clear supernatant subdivided into two aliquots which were incubated in a waterbath for 15 min, one at 37 °C and the other at 60 °C. Both were then analysed by the standard electrophoretic technique[6]. Every strain was classified on the basis of four replicated experiments. In the second procedure, for each fly to be analysed the homogenate (40 µl) was electrophoresed by the usual method. At the end of the electrophoretic run the starch gel was cut into two slices, which were transferred into thin plastic envelopes. One was dipped for 15 min in a waterbath at 37 °C, the other at 60 °C and then stained. By both these methods a given electrophoretic PGM band was classified in a clear cut way as a heat-resistant band (PGM R) or as a heat-sensitive band (PGM S) according to whether or not its PGM activity was detected after the treatment at 60 °C.

evidence that the heat-sensitivity behaviour of each PGM electrophoretic band depends on the corresponding *Pgm* structural allele is presented in Tables 2 (first procedure) and 3 (second procedure). It seems that the phenotype for heat sensitivity of each PGM band travels with the electrophoretic phenotype of the same band and is restricted to it (*cis–trans* effect). Therefore its genetic site(s) must be at the *Pgm* structural gene.

It was thus possible to search for a further polymorphic heterogeneity of the *Pgm* structural gene besides the electrophoretic one. For this purpose we determined the heat-sensitivity phenotype of common electrophoretic *Pgm* alleles derived from a laboratory and two natural populations. These alleles were tested, by the first procedure, in either the homozygous or heterozygous state as isogenic or TM2 balanced lethal stocks, respectively (Table 4). The previously homogeneous Pgm^{A} allele was split into two common isoelectrophoretic alleles, $Pgm^{\mathrm{A \cdot tr}}$ and $Pgm^{\mathrm{A \cdot ts}}$ in all samples examined. The Pgm^{B} allele seems so far to be homogeneous. Therefore a new type of structural heterogeneity of the *Pgm* gene has been discovered. This increases considerably the estimate of the degree of identified variability of this gene. For example, the degree of heterozygosity of the Castellaneta population increases from 0.17 based on the electrophoretic variation only (Pgm^{A}: 0.906; Pgm^{B}: 0.083; all the *Pgm* rare alleles: 0.011)[5] to 0.30.

Bernstein *et al.*[7] and Singh *et al.*[8] have reported common isolectrophoretic alleles of xanthinedehydrogenase in 11 species of the virilis group of *Drosophila* and octanoldehydrogenase in *D. pseudoobscura* with different thermostabilities. But they have not shown (1) that the heat-sensitivity phenotype is different in different allelic products of

Table 2 Testing the hypothesis that the heat-sensitivity of the PGM bands depends on alleles at the *Pgm* locus

Strains	Crosses ♂ × ♀	F$_1$ Ubx phenotypes	F$_1$ electrophoretic phenotypes† at 37 °C	at 60 °C
166 × 63	A^S × B^S		AB	—
68 × 63	A^R × B^S		AB	A–
103 × 55	A^R × B^S		AB	A–
55 × 103	B^S × A^R		AB	A–
138 × 63	A^S × B^S		AB	—
63 × 138	B^S × A^S		AB	—
246* × 63	D^R/A^R × B^S	Ubx + 232	AB	A–
		Ubx 192	DB	D–
11* × 63	C^R/A^R × B^S	Ubx + 215	AB	A–
		Ubx 241	CB	C–
63 × 11*	B^S × C^R/A^R	Ubx + 180	AB	A–
		Ubx 218	CB	C–
57* × 63	E^S/A^R × B^S	Ubx + 215	AB	A–
		Ubx 178	EB	—
126* × 63	C^S/A^R × B^S	Ubx + 203	AB	A–
		Ubx 174	CB	—
63 × 126*	B^S × C^S/A^R	Ubx + 195	AB	A–
		Ubx 213	CB	—
132* × 5	A^S/A^R × B^S	Ubx + 245	AB	A–
		Ubx 216	AB	—

*Balancer lethal stock; the TM2 third balancer chromosome carries simultaneously the ultrabitorax (Ubx^{130}) morphological dominant mutation and the Pgm^{A} heat-resistant allele.

†Determined on 50 adult-fly homogenates according to the first procedure described in the legend of Fig. 1.

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Table 4 Distribution of common *Pgm* alleles identified by combining electrophoretic and heat sensitivity determinations

Origin of the strains	Total genes examined*	Classification of the *Pgm* genes examined†			
		$Pgm^{A,tr}$	$Pgm^{A,ts}$	$Pgm^{B,tr}$	$Pgm^{B,ts}$
Laboratory	18	12	3	—	3
Castellaneta (Puglia)	61	45	4	—	12
Ranna (Sicilia)	67	45	2	—	20

Estimates of gene frequencies (obtained by multiplying the relative frequencies of heat-resistant and heat-sensitive alleles in each electrophoretic class by the gene frequencies of the corresponding electrophoretic alleles):

Castellaneta
$Pgm^{A,tr} = p_1 = 0.832$ } $P = 0.906$
$Pgm^{A,ts} = p_2 = 0.074$
$Pgm^{B,ts} = q = 0.083$
Pgm rare (pooled) $= r = 0.011$

Ranna
$p_1 = 0.907$ } $P = 0.947$
$p_2 = 0.040$
$q = 0.041$
$r = 0.012$

p, q and r are the estimates previously obtained by considering the electrophoretic phenotypes only[5]. p has been split further into p_1 and p_2 according to the relative frequencies of $Pgm^{A,tr}$ and $Pgm^{A,ts}$ alleles.

*Each of these genes was tested either as an isogenic line homozygous for that gene or as a balanced lethal stock in which that gene was heterozygous with a *TM2* chromosome carrying a *Pgm* allele with a known electrophoretic and heat-sensitivity phenotype.

†We propose to indicate the heat sensitivity of the *Pgm* electrophoretic alleles or phenotypes by adding to their name a superscript, *tr* and *ts* corresponding to thermoresistance and thermosensitivity, respectively.

Table 3 Absolute association between the Mendelian segregation of the electrophoretic and heat sensitivity phenotypes of four *Pgm* alleles

F_1 genotypes	Type of cross $\frac{A^R}{B^S} \times \frac{C^S}{D^R}$ No. of observed individuals	F_1 electrophoretic phenotypes*	
		at 37 °C	at 60 °C
$A^R\ C^S$	95	AC	A–
$A^R\ D^R$	76	AD	AD
$B^S\ C^S$	95	BC	—
$B^S\ D^R$	83	BD	–D

*Determined in single-fly homogenates following the second procedure described in the legend of Fig. 1.

the structural gene studied in appropriate heterozygotes, and, as a necessary consequence of this, (2) that the heat-sensitivity phenotype is transmitted together with a certainly identified structural phenotype (for example, electrophoretic). Therefore their evidence seems to be incomplete because it has not been excluded that the observed heat sensitivity phenotypes were due to a different gene. For example, the thermostability of the autosomal enzyme adeninephosphoribosyltransferase, in man is greatly influenced by the sex-linked hypoxanthineguaninephosphoribosyltransferase (HGPRT) deficiency[9]. Nevertheless it seems likely that those alleles exist because situations such as HGPRT deficiency are probably not common and, on a theoretical basis, many structural genes are expected to have polymorphic isoelectrophoretic alleles.

This work was supported by a grant of the National Research Council.

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Received November 3; accepted December 29, 1975.

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Nude mice from homozygous nude parents show smaller PFC responses to sheep erythrocytes than nude mice from heterozygous mothers

THE congenitally athymic mouse which is homozygous for nude (nu/nu), compared with the phenotypically normal heterozygote (+/nu) or the normal mouse (+/+) is severely deficient in its plaque-forming cell (PFC) response to sheep erythrocytes (SE)[1,2]. The minimal response of the nude is generally attributed to a deficiency of thymus-derived helper cells which are essential for a full PFC response to thymus-dependent antigens such as SE[1–3]. The amount of helper activity (T cell or T-cell-like) that nude mice may receive from their mothers is of concern in several laboratories[4–9], since most nude mice obtained routinely are born of phenotypically normal (+/nu) mothers that have T cells. There is debate about the relative importance of T-cell precursors observed in nude mice[10], which may be stimulated to express T-cell antigens by soluble factors[11], and the equally important possibilities that nudes may receive T cells and/or soluble factors *in utero* from their normal +/nu mothers or from their +/nu or +/+ littermates or from the colostrum of +/nu mothers[12]. The results of studies designed to resolve this issue remain equivocal[4–13]. There is a need[12] for studies with nude mice born of homozygous nude parents (nu/nu ♀ × nu/nu ♂) and raised by nude mothers to minimise the possibilities of transfer of functionally mature T cells *in utero* or the transfer of T cells or T-cell-like factors by the colostrum. So far this criterion has been difficult to meet since nude mothers from most common mouse strains were poorly fertile or could not raise their young[14].

In initial experiments using NIH strain nude mice, we compared the 4-d PFC responses of nude mice of the following parentages, nude (nu/nu) mothers × nude (nu/nu) fathers (type I), heterozygous (+/nu) mothers × nude fathers (type II), and type I offspring, foster nursed by +/nu mothers (type III). The breeding and husbandry of these nude offspring is to be detailed elsewhere. Briefly, the NIH nude mouse colony was established in the summer of 1972. The initial stock was received on a BALB/c background. The *nu* gene was next established into the N: NIH(S) outbred stock of mice, which has a very high reproductive performance. Breeding at this stage was conventional, that is, heterozygous (nu/+) females with homozygous (nu/nu) males yielding 50% homozygous offspring. In the second stage the nu/nu progeny was established and maintained in a specific pathogen-free (SPF)

Table 1 PFC responses of NIH strain nude mice from nude mothers and heterozygous mothers to sheep erythrocytes

Type of nude		Type of mothers		Cells per spleen	PFC	
		Born to	Nursed by	(× 10^6)	per 10^6 cells	per spleen
I	Mean ± s.e. for 10 mice	nu/nu	nu/nu	126 ± 7	1.4 ± 0.09	174 ± 11
II	Mean ± s.e. for 10 mice	+/nu	+/nu	197 ± 16	12.8 ± 3	2,652 ± 701
III	Mean ± s.e. for 6 mice	nu/nu	+/nu	153 ± 9	6.1 ± 0.5	932 ± 105

Plaque-forming cell (PFC) responses to SE of type I nude mice (homozygous nude mothers) and of type II nude mice (heterozygous +/nu, phenotypically normal mother) and type III (homozygous nude mothers, but foster nursed by +/nu mothers) are compared. The fathers in all cases were homozygous nudes. Each mouse was injected intravenously with 4 × 10^8 SE and the direct PFC count per spleen or per 10^6 acid-resistant splenocytes was estimated for individual mice by the localised haemolysis-in-gel assay described before[15,16]. Cells per spleen were estimated electronically in the Fisher Autocytometer-II. Thymus tissue was not visible in nude mice of this study.

Table 2 *In vitro* PFC responses to sheep erythrocytes by splenocytes from NIH strain nude mice and the effect of dibutyryl cyclic AMP

Type of nude splenocytes cultured		Dibutyryl cyclic AMP	Splenocytes recovered after 4 d in culture (× 10^6)	PFC detected after 4 d of culture	
				per 10^6 recovered splenocytes	per culture
I	Mean ± s.e. for 10 seriate cultures	–	10.6 ± 0.3	3.7 ± 1.8	42 ± 6
I	Mean ± s.e. for 5 seriate cultures	+	12.5 ± 0.8	32.6 ± 5.6	417 ± 95
II	Mean ± s.e. for 10 seriate cultures	–	9.6 ± 0.4	16.5 ± 2.5	153 ± 17
II	Mean ± s.e. for 5 seriate cultures	+	12.6 ± 3.2	24.2 ± 1.6	371 ± 17

Comparison of *in vitro* plaque-forming cell (PFC) responses of dispersed spleen cells from type I nude mice and type II nude mice to sheep erythrocytes (SE) after 4 d in culture. The effect of dibutyryl cyclic AMP (1.5 μM per culture of N^6, O^2-dibutyryl adenosine-3′-5′-cyclic phosphate, Calbiochem) on similar cultures are compared. Type I splenocytes (31 × 10^6 per culture) and type II splenocytes (29 × 10^6 per culture) were cultured in the presence of 2 × 10^6 SE as antigen in modified Marbrook chambers as described before[16]. The number of cells and the number of PFC were estimated as stated in the legend for Table 1.

environment by hysterectomy and foster nursing on previously established germ-free (GF) mice. This environment consists of an SPF open room barrier facility, requiring that all supplies which enter be sterile. Movement of personnel who enter is closely controlled and personnel wear sterile garments, including face masks and gloves. The rooms and animals are monitored for infectious bacteria and viruses. No evidence of infectious viruses has been found. This includes scrutiny for mouse hepatitis virus (MHV), to which nude mice are extremely susceptible In these conditions, survival of homozygotes to 28 months was achieved. In the third stage, 30 pairs of homozygous matings were established as soon as animals were available. It was important to scrutinise the colony continuously and to separate individuals suggestive of sterility and lactation-failure. By these procedures, the total number of homozygotes weaned from homozygous mothers is not much smaller than that for heterozygous mothers. Our experience is that homozygous mothers weaned an average of 9.2 offspring during their reproductive life compared with an average of 12 by heterozygous mothers.

The PFC responses of type I nudes (PFC/spleen) were 15 times less than those of type II nudes (Table 1). The difference estimated on a per cell basis (per 10^6 cells) was approximately tenfold. Type III nudes, which were produced by foster nursing type I nudes on T-cell positive, +/nu mothers showed PFC responses that were four to five times greater than type I nudes (Table 1).

In spleen cell cultures (Table 2), type I nude mouse PFC responses were similarly less than those of type II nudes. Dibutyryl cyclic AMP which exerts a T-cell like helper effect[15] augmented tenfold the PFC culture responses of type I spleen cells, raising them to approximately the control level of type II cultures not exposed to the compound.

These data show that nude mice from homozygous nude parents are more immunologically deficient with respect to their ability to activate antibody-forming cells than the more conventionally bred nude mice derived from heterozygous mothers. The greater PFC responses observed in nude mice borne by nude mothers, but nursed from birth by heterozygous foster mothers are consistent with the suggestion that athymic nude mice receive T-cell help from heterozygous mothers. Further clarification of the possible roles of both T cells and soluble T-cell-like factors awaits isolation and identification of the cells and factors. The status of T-cell precursors in nude mice described here remains to be evaluated.

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Suppression of adoptive antibody responses by addition of spleen cells from agammaglobulinaemic chickens "immunised" with histocompatible bursa cells

THE report that T cells from patients with common variable hypogammaglobulinaemia can suppress immunoglobulin synthesis by B cells *in vitro*[1] implies that some forms of hypogammaglobulinaemia could, at least in part, be maintained by a patient's own T cells. Blaese *et al.*[2] described a phenomenon of "infectious agammaglobulinaemia" in line 6 chickens (USDA Regional Poultry Research Laboratory, East Lansing) which seems to represent an ideal laboratory model for the human disease. Transfer of agammaglobulinaemia with spleen cells has now also been observed consistently in strain FP and SC chickens[22]. The mechanism whereby recipients of cells from an agammaglobulinaemic donor are rendered agammaglobulinaemic is, however, difficult to analyse in such a long term *in vivo* system. Thus, further examination of the phenomenon would be greatly facilitated by a short term assay to allow better identification of the target cell.

We describe here a transfer system in which the suppressor activity of spleen cells from agammaglobulinaemic chickens on the adoptive immune response of normal spleen or bursa cells is assayed in neonatal irradiated recipients. The target system used is the antibody response to *Brucella abortus* (BA), which is thymus independent[3] and readily detected in recipients within 1 week of bursa cell transfer[4]. Using this 1-week assay, we have found that spleen cells from bursectomised (BX) donors interfere with an adoptive anti-BA response only when they are taken from BX animals previously injected with bursa cells.

Donor and recipient birds were of strains FP (B15/B21) and SC (B2/B2) (Hy-Line International). BX birds were obtained by *in ovo* injection of 3.75 mg testosterone propionate (Elkins–Sinn) on day 11 of gestation, followed by intraperitoneal injection of 4 mg cyclophosphamide (Mead–Johnson) on the day of hatching, and 3 mg on each of the 2 subsequent days[5]. Sera of BX birds were tested for Ig by double diffusion in agar, using rabbit antiserum to chicken Ig to detect readily Ig at 1% normal 8-week serum level.

Some BX birds of the FP strain were injected intravenously with 2×10^7 male bursa cells 6–13 d before being killed. For the SC strain, the injection schedule was more varied (Table 3). Chicken Ig (mixture of IgM and IgG, prepared as described[6]) was injected subcutaneously with Freund's complete adjuvant (FCA).

Table 2 Effect of pretreatment of suppressor cells on inhibition of adoptive immune response, FP strain

Bursa-immune BX spleen (4×10^7)*	Pretreatment of BX spleen†	Mean $\log_2$ agglutinin serum titres in recipients ± s.e.‡ Day 7	Day 14
−		14.4 ± 0.8	14.0 ± 0.9§
+	None	0.3 ± 0.0	4.9 ± 2.1
+	Mitomycin	4.1 ± 0.4	8.3 ± 2.0
+	NRS+C	0.0 ± 0.0	2.8 ± 2.0
+	Anti-T+C	11.8 ± 1.3	11.5 ± 1.7§

*On day 0, recipients injected intravenously with 4×10^7 bursa cells from 6-week-old donors, 2×10^8 BA, with or without 4×10^7 viable spleen cells from a BX donor; this BX donor received 2×10^7 bursa cells intravenously on day −13 (experiment 4).

†Mitomycin (50 μg ml^{-1}) 15 min at 37 °C, anti-T or normal rabbit serum (1/90) and C (1/18) 37 °C for 1 h.

‡Anti-BA agglutinin titres determined on sera taken on day 7; recipients were reinjected with BA on day 7 and bled on day 14.

§$0.2 < P < 0.3$.

Recipients were either injected with 4 mg of cyclophosphamide intraperitoneally on the day of hatching and treated with 500 r. whole-body ^{138}Cs γ irradiation (Gammator M. Radiation Machine Co.) or they received 500 and 800 r. on days −2 and −1, respectively. On the day of transfer, day 0, donor cell suspensions were prepared in 0.165 M phosphate-buffered saline containing 0.2% bovine serum albumin and, in the case of bursa cell suspensions, heparin (2 μg ml^{-1}). BX spleen cells (6.7×10^6 cells per ml) were treated with rabbit anti-T serum[7] or normal rabbit serum (NRS) (1/90) and young guinea pig complement (C) (1/18) for 1 h at 37 °C. Treatment with mitomycin was for 15 min at 37 °C at 10^7 cells per ml. BX spleen donor cells (4×10^7 viable) and target bursa or spleen cells (4×10^7) were mixed just before injection into leg veins of recipients (six to eight per group). The BA antigen was also added immediately before injection (approximately 2×10^8 washed dead bacteria). Anti-BA agglutinin titres were determined on sera taken 1 week after transfer, and in some cases on sera taken at 2 weeks after a second BA injection on day 7. The day-14 sera (minimum of four chickens per group) were also analysed after exposure to 0.1 M mercaptoethanol in order to measure 7S antibody titres[8]. Experiments lasting 2 weeks were possible as control animals injected with antigen alone on days 0 and 7 gave no appreciable responses.

The results in Table 1, obtained in the FP strain, show

Table 1 Ability of spleen cells from BX chickens to inhibit adoptive antibody production by bursa or normal spleen cells, FP strain

Responding cell type (4×10^7)	BX spleen added* (4×10^7)	BX donor injection†	Mean $\log_2$ agglutinin serum titres in recipients ± s.e.‡ Expt 1 Day 7	Expt 2 Day 7	Expt 3 Day 7
Spleen	−		5.0 ± 0.9	5.3 ± 1.7	
Spleen	+	Nothing	5.3 ± 0 8	5.4 ± 1.2	
Spleen	+	Bursa	1.6 ± 0.5	2.3 ± 0.6	
Spleen	+	Ig		5.8 ± 1.1	
Bursa	−			11.3 ± 0.3	12.0 ± 0.7
Bursa	+	Nothing		10.9 ± 0 5	10.8 ± 0.9§¶
Bursa	+	Bursa		1.6 ± 0.8	5.0 ± 0.3§¶
Bursa	+	Spleen			8.7 ± 0.2
Bursa	+	Thymus			9.5 ± 0.9¶
Bursa	+	Ig		10.3 ± 0 3	

*On day 0, recipients injected intravenously with responder cells from 6–9-week-old donors, BA (2×10^8), with or without spleen cells from agammaglobulinaemic chickens (3–4 months old).

†"Immunisation" of BX donors with bursa, spleen or thymus cells injected intravenously as follows: experiments 1 and 2, 2×10^7 cells on day −6 or −7; experiment 3, 2×10^6 cells on day −13. "Immunisation" with chicken Ig was on day −7, 200 μg with CFA injected subcutaneously.

‡Anti-BA agglutinin titres determined on sera taken on day 7.

§$P < 0.001$.

¶$0 3 < P < 0.4$.

Table 3 Ability of spleen cells from BX chickens to inhibit adoptive antibody production by bursa cells, SC strain

Cell type added (4×10^7)*	BX donor injection†	Mean $\log_2$ agglutinin serum titres in recipients ± s.e.‡				
		Expt 5	Expt 6	Expt 7	Expt 8	
		Day 7	Day 7	Day 7	Day 7	Day 14
None		9.8±0.4	14.2±0.3	9.3±2.1	11.7±0.8§	15.8±2.0 (7.3±1.1)¶
BX Spleen	Nothing	11.5±0.3	14.3±1.2			
BX Spleen	Bursa, once	10.5±0.2	13.5±0.4	4.8±0.4	10.4±1.0	9.8±2.1 (2.8±0.9)
BX Spleen	Spleen	10.7±0.5				
BX Spleen	Bursa, twice				6.5±0.7§	7.7±2.0 (3.0±0.6)¶

*On day 0, recipients injected intravenously with bursa cells from 6–9-week-old donors and BA (2×10^8), with or without spleen cells from agammaglobulinaemic chickens (3–4 months old).

†"Immunisation" of BX donors with bursa or spleen cells administered as follows: experiment 5, 2×10^7 cells intravenously day −7; experiment 6, 2×10^7 cells intravenously on day −21; experiment 7, 2×10^7 cells intravenously on day −89; experiment 8, 2×10^7 cells with CFA subcutaneously on day −7 ("once") and 2×10^7 cells intravenously on day −52 ("twice")

‡Anti-BA agglutinin titres determined on sera taken on days 7 and 14.

§$P<0.001$.

¶Titres remaining after incubation of sera with 2-mercaptoethanol are in parentheses. $P<0.01$.

that BX spleen, injected together with either bursa or spleen cells from 6–12-week-old normal chickens, did not inhibit responsiveness of normal cells. But when spleen cells were taken 1–2 weeks after intravenous injection of 2×10^7 bursa cells into 3–4-month-old BX chickens, they caused a considerable reduction of the adoptive antibody response (Tables 1 and 2). Injection of fewer bursa cells (2×10^6, experiment 3) was somewhat less effective but still seemed to immunise the BX donors much better than did 2×10^6 spleen or thymus cells. The lack of immunisation with thymus cells suggested that bursa cell-specific antigen rather than antigen determined by minor histocompatibility loci was responsible for the immunisation effect. The age of the BX chicken at which immunisation with bursa cells could be effected was not determined precisely, but in one experiment (not in Tables 1 and 2) such an immunisation proved ineffective in a 2-month-old BX chicken. Other attempts to immunise BX chickens were made using chicken-Ig in CFA; these had no effect (experiment 2, Table 1). This suggested that Ig molecules, as present in serum, did not constitute the moiety against which BX spleen cells were reacting. The fact that the inhibitory effect on bursa and spleen cells occurred in recipients which obviously had circulating maternal IgG (ref. 9) supports this conclusion. Further efforts towards identification of the bursa cell antigen included an experiment (not in Tables 1 and 2) in which immunisation with 4×10^6 paraformaldehyde-treated (30 min at 4 °C, $5+10^6$ cells and 0.1 g ml^{-1}) bursa cells failed.

Since bursa cells from young chickens generally give a higher adoptive response to BA than do spleen cells[4], experiments to characterise the effector cell further were performed with bursa cells as the target. "Bursa-immune", BX spleen cells still reduced the immune response after exposure to mitomycin ($P<0.05$ compared with $P<0.01$ for untreated cells, Table 2). In addition, the effector cell in the BX spleen was largely eliminated by treatment with rabbit anti-T but not by NRS+C, suggesting its T-cell nature (Table 2). Thus, the BX spleen cell responsible for this phenomenon seems to be a T cell which, after recent immunisation with its target cell, does not need to proliferate to inhibit B cells. For prolonged maintenance of B-cell suppression in recipients, however, the untreated or NRS-treated BX spleen cells were more effective than were the mitomycin-treated (compare day 14 with day 7 results, Table 2).

Several of the recipients in experiment 4 which showed no anti-BA on day 14 after transfer of bursa cells + "bursa-immune" BX spleen still failed to produce anti-BA 4 weeks later and had no detectable Ig in their sera. This latter observation suggests strongly that the phenomenon studied here is analogous to that described as "infectious agammaglobulinaemia" by Blaese *et al.*[2] and Palladino *et al.*[22].

As mitomycin-treated cells were effective, an experiment was performed in which a 5 and a 9-month-old FP chicken —birds which were undergoing and which had undergone complete involution of the bursa—were injected with bursa cells and used as spleen donors 1 week later. We reasoned that natural involution of the bursa might be caused by an immune response to bursa antigens occurring at a later age. Mitomycin was required since adult spleen cells would otherwise transfer their own anti-BA response. The mitomycin-treated cells from these spleens did not reduce the ability of bursa cells to give adoptive anti-BA responses. This approach, therefore, was discontinued.

Results with the SC strain were much less striking than those with the FP strain (Table 3). Immunisation with neither bursa nor with spleen cells caused a rapid appearance of bursa-inhibitory activity in the spleen cells of agammaglobulinaemic donors. A longer interval after immunisation with bursa cells may have been needed, as suggested by the results of experiments 7 and 8. The later (day 14) bleedings in experiment 8 indicated that both 19S and 7S anti-BA antibody formation were suppressed. There was also a decreased response in a later bleeding taken in experiment 6 (not shown in Table 3). In this experiment, the day 14 data were very similar to those in experiment 8, but loss of several control recipients precluded appropriate analysis.

Experiments by Palladino *et al.*[22] have also shown that transfer of agammaglobulinaemia was obtained more readily in FP strain than in SC strain, although inhibition of antibody formation could be demonstrated in both. It was further observed in our laboratory that FP chickens give far better delayed hypersensitivity reactions to protein antigens than do SC chickens, suggesting a much more responsive T-cell system in the FP strain. The results reported here suggest that the difference between the FP and SC strains noted by Palladino *et al.* was due to a variation in T-cell reactivity and not to a difference in B-cell mass, since a uniform dose of target cells was used in our experiments. The more readily demonstrable BX spleen immunity to bursa cells in the FP strain may also be related to the relative ease in the FP compared with the SC strain with which agammaglobulinaemia can be obtained (unpublished).

The antigen to which the splenic T cells of BX chickens respond is unknown. Candidates include (1) Ig in a form peculiar to the B-cell surface (IgD analogue)[10]; (2) viral antigen which might normally be expressed on bursa and B cells only[11,12], and (3) any other surface antigen unique to B cells[7,13] in the resting and/or activated state. This is reminiscent of the conclusion of Jacobson *et al*[14] that the target antigen on cells in long term, T-cell mediated[15] allotype suppression could not be Iglb in the same form as it is in the circulation. This phenomenon in mice, applying to a subpopulation of B cells, seems strikingly similar to that of transferred agammaglobulinaemia in chickens, where all B-cell function becomes suppressed. It has also been suggested that suppressor cells have a role in the maintenance of long term allotype suppression of rabbits[16].

Since the suppression of antibody production in our studies as well as in those of Palladino *et al.*[22] and Blaese *et al.*[2] results in agammaglobulinaemia of recipients, the phenomenon is not likely to be antigen specific. Tada and Takemori[17], however, have noted that even in studies on antigen-specific T-cell suppression of the immune response, the susceptibility of B cells to the specific suppressive influence seems to differ depending on the stage of differentiation of B cells and/or on the class of Ig they synthesise.

The lack of tolerance to an organ which was removed during ontogeny and reintroduced later in development was described originally for the hypophysis in tadpoles[18]. The observation that immunity to bursa cells can be induced in chickens deprived of bursa early in ontogeny may similarly be ascribed to a lack of tolerance to tissue (bursa)-specific antigens. The development of immune T cells, in this case called suppressor cells, may then represent the normal mechanism of cytotoxic cell development such as is observed to minor transplantation antigens[19] and to tumour antigens[20,21].

We thank Melvin Bell and Pedro Sanchez for assistance. *B. abortus* was given by Dr C. E. Watson, USDA National Animal Diseases Laboratory, and mitomycin C was obtained through the courtesy of Dr H. B. Wood Jr, National Cancer Institute. This work was supported by grants from the US Public Health Service.

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Received November 3; accepted December 12, 1975.

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Modification of fibrosarcomas and squamous cell carcinomas of mouse skin using various tissue extracts

RECENT attention has focused on the possibility of utilising (or mimicking) aspects of mitotic processes in the control of cancer. Bullough, Rytömaa and others used preparations of mitosis inhibiting proteins (chalones) to produce remissions (total in some cases) in a number of tumour tissues[1–3]. These chalone preparations were shown to be tissue specific. Only epidermal chalone worked on epidermal tumours. Liver extracts prepared in the manner of epidermal chalone extracts gave no remissions in epidermal tumours[4]. Melanocyte chalone was necessary for remissions in melanomata[5] and granulocyte chalone was successful in the treatment of chloroleukaemia[6]. We report here remissions resulting from the treatment of chemically-induced fibrosarcomas and squamous cell carcinomas of the skin in mice using extracts of various mouse tissues. We also report the presence of mitosis-inhibiting protein in these extracts.

Excised whole skin, liver or tumour, frozen in liquid N_2 and minced, was homogenised with 0.14 M NaCl–0.14 M phenol in a Waring Blender for 15 h at 4 °C. After centrifugation for 2 h at 2,500 r.p.m., the supernatant, combined with 5% sodium dodecyl sulphate in 45% EtOH, was stirred for 24 h at 4 °C, then centrifuged (1 h, 2,500 r.p.m.). The resulting supernatant was transferred to a new tube and again centrifuged (1 h, 2,500 r.p.m.). The final supernatant was combined at 4 °C with an equal volume of cold 95% EtOH and the precipitate collected.

One portion of the extract obtained from whole skin was treated with α-chymotrypsin to remove protein. A second portion was treated with deoxyribonuclease and ribonuclease to remove nucleic acids. Analysis, using modified Dische techniques[7], of the extracts showed that the percentage DNA tripled after chymotrypsin treatment relative to the untreated skin extract and, after deoxyribonuclease–ribonuclease treatment, was one-sixth that of the untreated skin extract. Each of the five extracts (the three tissue extracts and the two treated skin extracts) were suspended in sterile 0.9% saline (100 μg extract per 0.05 ml) for injection.

Fibrosarcomas and squamous cell carcinomas were induced in male BALB/c mice through three injections each

Table 1 Percentage remissions for all animals

Group	No. of mice	Percentage remissions 1 month*	2 months*	3 months*
(1) Treated with unreacted skin extract	28	53	†	†
(2) Treated with unreacted skin extract	40	48	28	5
(3) Treated with liver extract	19	47	16	21
(4) Treated with skin extract reacted with chymotrypsin	14	21	29	17
(5) Treated with skin extract reacted with nucleases	18	56	17	17
(6) Treated with tumour extract	12	0	0	33

*Percentage of each group still in remission 1, 2, or 3 months after treatment was started

†Tumour size measurements not made.

Table 2 Percentage remissions for animals with squamous cell carcinomas

Group*	No. of mice	Percentage remissions		
		1 month†	2 months†	3 months†
(1)	15	73	‡	‡
(2)	16	88	56	50
(3)	12	67	25	8
(4)	6	50	67	50
(5)	8	75	25	0
(6)	7	0	0	57

*Groups are the same as in Table 1.

†Percentage of each group still in remission 1, 2 or 3 months after treatment was started.

‡Tumour size measurements not made.

of 100 μg (in 0.05 ml linseed oil) of either 9,10-dimethylbenzanthracene or benz(*a*)pyrene spaced over a period of 2 weeks. Tumours were developed for 3 months and then the animals were screened. Those with very large tumours (larger than about 10 cm^3) and those showing no tumour development were excluded (in the environmental control group no evidence of spontaneous tumour formation was observed). Tumours were then inoculated with 5–100 μg injections (over a 1-month period) of one of the extracts. Effects of the extracts on tumours were monitored by size measurements. Selected animals were killed and tissue samples examined by the Department of Pathology, College of Medicine, University of Kentucky.

Untreated fibrosarcomas and squamous cell carcinomas showed no spontaneous remissions. Injection into tumours of extract or of 0.9% saline produced uniform minimal mechanical damage. The injection of 0.9% saline alone produced no remissions. Subcutaneous injection of tumour extract or of skin extract into healthy animals produced no apparent effects, establishing the absence of any general cytotoxic material in those extracts.

In two separate experiments, with separate preparations of the whole (untreated) skin extract and with separate animal populations similar effects on tumours were noted (Tables 1, 2 and 3). For all animals in these groups (both those with fibrosarcomas (FS) and those with squamous cell carcinomas (SCC)) the percentage of animals showing remissions one month after initiation of treatment with skin extract were 53% (n=28) and 48% (n=40). For animals with SCC, the percentage remissions were 73% (n=15) and 88% (n=16). For animals with fibrosarcomas, the percentage remissions were 31% (n=13) and 21% (n=24). The smaller percentages observed for animals with FS could result from the difficulty in reaching all parts of the tumour with the extract. FS were larger than the SCC, and may have been too large to treat in some cases—sizes at treatment initiation ranged up to 10 cm^3. The percentages fall after the first month, suggesting that the effect of the skin extract may be temporary and/or that a longer period of treatment may be necessary. The animals with continuing remissions had smaller tumours initially.

Table 3 Percentage remissions for animals with fibrosarcomas

Group*	No. of mice	Percentage remissions		
		1 month	2 months	3 months
(1)	13	31	‡	‡
(2)	24	21	8	0
(3)	7	14	0	0
(4)	8	0	0	0
(5)	10	25	10	10
(6)	5	0	0	0

*Groups as in Table 1

†Percentage of each group still in remission 1, 2 and 3 months after treatment was started.

‡Tumour size measurements not made.

The liver extract produced remissions in 47% (n=19) of all mice treated with liver extract (Table 1). The liver extract produced remissions in 67% (n=12) (Table 2) of the animals with SCC and 47% (n=7) (Table 3) of the animals with FS. If the extraction of skin and liver yield the same products, then these percentages for the liver extracts, which are similar to those for the untreated skin extract, indicate a lack of tissue specificity in the extract actions. Tumour extract produced delayed remissions only (0% 1 and 2 months after treatment initiation for both FS and SCC; 33% (n=12) for all animals 3 months after treatment; 57% (n=7) 3 months after for SCC; and 0% (n=5) after 3 months for FS) (Tables 1, 2, 3). The reason for these delayed remissions is uncertain, but it may be that the remission-producing protein in the tumour extract is present in a decreased amount.

Treatment of tumours with skin extract reacted with nucleases produced remissions in percentages (56% (n=18) for all animals; 75% (n=8) for SCC; 25% (n=10) for FS) (Tables 1, 2, 3) similar to those observed following treatment with the unreacted skin extract. Skin extract treated with chymotrypsin, however, yielded markedly reduced remission percentages (21% (n=14) for all animals; 50% (n=6) for SCC, 0% (n=8) (Tables 1, 2, 3), suggesting that the active component in the skin extract is protein.

An experiment measuring the uptake of ^{3}H-thymidine into cultures of mouse ear in Hank's medium demonstrated the presence of a mitosis-inhibiting substance in untreated skin extract. This material may or may not be the same material which affects tumours.

We wish to thank the American Cancer Society and the Department of Chemistry, University of Kentucky for their support of this work.

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Received October 3, 1975; accepted January 19, 1976.

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Mediation of retinoic acid-induced growth and anti-tumour activity

VITAMIN A (retinol) is a nutrient essential for vision, growth, reproduction and proper differentiation of epithelial tissue[1]. Some retinol is oxidised to retinoic acid (vitamin A acid) *in vivo*[2], but if an animal is provided with retinoic acid in place of dietary retinol, it can only partially substitute for the missing retinol; vision[3] and reproduction are impaired[4]. Sporn *et al.*[5] have described two systems for evaluating the activity of retinol, retinoic acid, and their analogues *in vitro*. One measures the ability of such compounds to promote growth by determining RNA, DNA and protein levels after addition of the test compound to epidermal cell cultures derived from newborn mouse skin; the other measures ability to reverse metaplasia of the epithelial cells of trachea explanted to organ culture from vitamin A-deficient hamsters. Retinoic acid is active in both systems.

In addition, retinoic acid and an aromatic analogue of the acid have been shown to reverse metaplasias of mouse skin induced by application of carcinogenic drugs and expressed as papillomas and tumours[6]. The molecular mechanism(s) by which retinoic acid or retinol control growth and differentiation, as well as the mechanism of their carcinostatic ability, is still unknown but may involve the specific cytoplasmic binding proteins we have discovered for retinol and retinoic acid The cellular retinol-binding protein binds retinol *in vitro*[7] and *in vivo*[8], is present in many tissues, and exhibits binding affinities for *cis* isomers of retinol which parallel the growth-promoting activity of these isomers in the whole animal[9]. The cellular retinoic acid-binding protein binds retinoic acid *in vitro* with high specificity and is present in normal rat[8] and human organs[10] as well as some human cancers[11]. The two proteins have been separated physically and purified 1,000-fold from rat testis[8]. There has also been a preliminary report of a retinoic acid-binding protein in embryonic chick skin[12].

In our investigations into the mechanism(s) of action of retinol and retinoic acid we have examined newborn mouse skin, hamster trachea, and chemically induced papillomas of mouse skin for the presence of the cellular retinoic acid-binding protein. In addition, using extracts of mouse skin papillomas, human breast tumour, and a 1,000-fold purified preparation from rat testis as sources for the binding protein, we have examined its relative affinity for some ring analogues of retinoic acid (Fig. 1). In all cases we find that the binding characteristics of the protein towards the analogues correlate with the activity of the compounds in the assay systems of Sporn *et al.* as well as with reported tumour-inhibiting activity.

Newborn mouse skin, adult mouse skin, mouse skin papillomas induced by treatment with 7,12-dimethylbenz(*a*) anthracene and croton oil[6], hamster trachea, and human breast tumour were homogenised in 0.05 M Tris-HCl, *p*H 7.1, and soluble extracts prepared as described previously[8]. A preparation of cellular retinoic acid-binding protein, purified 1,000-fold from rat testis as described before, was also used[8].

The binding protein was detected and its affinity to various analogues was determined as described earlier, using radioactive retinoic acid and sucrose gradient centrifugation[8]. The binding affinity was assessed by competition experiments and expressed as percentage inhibition (percentage decrease) of binding of radioactive all-*trans* retinoic acid observed when the competing analogue was present in a 100-fold molar excess over the ^{3}H-retinoic acid[8]. Details of the determination are presented in Table 1.

Analysis of extracts of newborn mouse skin and of hamster trachea revealed a peak of radioactivity in the 2S region of the sucrose gradient, indicating the presence of cellular retinoic acid-binding protein. It was also established that the mouse skin papillomas have a high level of the binding protein, quantitated as described previously[8], (860 pmol of ^{3}H-retinoic acid bound per g wet tissue) compared with control skin of mice of comparative age (56 pmol ^{3}H-retinoic acid bound per g of wet tissue). This difference may be due to an increase in the numbers of a cell type which normally contains this binding protein, or due to an altered expression of the genome. We have also found that several malignant human tumours (breast and lung) contained the cellular retinoic acid-binding protein but it was not detectable in normal tissue from the same organ of the same patient[11].

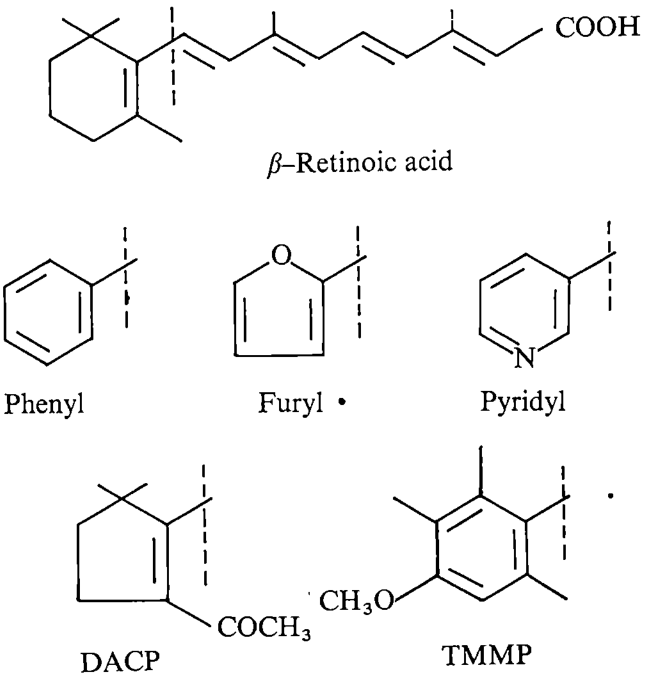

Fig. 1 Structures of β-retinoic acid and analogues with ring modifications.

The presence of this binding protein suggests that the protein might mediate the action of retinoic acid. This contention is strengthened by the affinity which the cellular retinoic acid protein exhibits, independent of the source, towards analogues of retinoic acid. Both the dimethylacetyl-cyclopentenyl (DACP) and trimethyl-methoxyphenyl (TMMP) analogues of retinoic acid are effective competitors for the binding of retinoic acid (Table 1). The phenyl, furyl and pyridyl analogues were less effective competitors in the order listed, with presumably decreasing affinities. These results correlate well with the ability of these compounds to promote growth in epidermal cell cultures derived from mouse skin and to reverse the metaplasia of hamster trachea induced by vitamin A deficiency as shown by Sporn *et al.*[5]. These authors noted that DACP and TMMP had marked activity in their systems, whereas the phenyl analogue had minimal activity and the furyl and

Table 1 Percentage inhibition of binding of all-*trans* ^{3}H-retinoic acid to cellular retinoic acid-binding protein by analogues of retinoic acid

Compound	Rat testis binding protein (%)	Mouse papilloma binding protein (%)	Human breast tumour binding protein (%)
All-*trans* retinoic acid	100	100	100
DACP analogue	100	100	100
TMMP analogue	100	100	100
Phenyl analogue	42	36	36
Furyl analogue	34	16	24
Pyridyl analogue	22	0	0

The preparations, made to 0.3 ml with 0.05 M Tris, *p*H 7.1, were incubated with 1×10^{-7} mmol radioactive all-*trans* retinoic acid (1.37 Ci mmol^{-1}) in the absence and presence of 1×10^{-5} mmol unlabelled retinoic acid analogues shown in Fig. 1. Each compound was added in 5 µl ethanol containing 1 mg ±-α-tocopherol ml^{-1}. Incubation mixtures whose protein concentration was below 1 mg ml^{-1} also contained 0.3 mg myoglobin and 0.3 mg purified bovine serum γ-globulin fraction. After incubation in the dark at 4 °C for 5 h, 0 2 ml of a charcoal–dextran solution was added with agitation After 5 min the charcoal–dextran was removed by centrifugation and 0.2 ml of the supernatant liquid was layered on a 5–20% linear sucrose gradient and centrifuged at 148,000*g* for 20 h. The gradients were collected in twenty fractions and the radioactivity of each fraction determined. Binding to the cellular retinoic acid-binding protein was indicated by a peak of radioactivity in the 2S region of the gradient.

pyridyl analogues seemed to be devoid of activity, compared with retinoic acid.

The apparent affinity of the cellular retinoic acid protein towards the TMMP analogue is of particular interest in that administration of the ethyl ester of this compound, or retinoic acid itself, is effective in preventing induction of mouse skin papillomas. These compounds can also cause the disappearance of the papillomas once induced[6].

The DACP analogue of retinoic acid is reported to block the ability of 3-methylcholanthrene to induce hyper- and metaplasia of prostate epithelium[13]. We have not examined this tissue for presence of the binding protein but we note, as have others[12], that the DACP analogue binds effectively to the protein.

It has been suggested that the reversal of metaplasias induced by vitamin A deficiency and by chemical carcinogens by retinoic acid and its TMMP analogue may be similar phenomena[14] The striking similarity between the binding affinity of the cellular retinoic acid protein for retinoic acid analogues and the growth-promoting as well as anti-tumour activity of these analogues suggests, circumstantially, that the action of retinoic acid in these instances could be mediated by this cellular protein.

Furthermore, the results reported here, as well as those reported earlier for cellular retinol binding protein[8], suggest that evaluating analogues of retinol and retinoic acid for their ability to bind to cellular retinoic acid or retinol-binding protein may be a useful preliminary screening step to identify those with potential growth or anti-tumour activity. Conversely, screening tumours for the presence of binding protein may indicate possible responsiveness of the tumours to treatment by such compounds

We thank Dr P. W. Trown for samples of mouse skin papillomas and untreated control skin. We also thank Hoffmann-La Roche for providing the analogues of retinoic acid and ^{3}H-retinoic acid. This work was supported by grants from the US Public Health Service.

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Received October 31; accepted December 12, 1975

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Nutrient-dependent arrest of fibroblast growth is partially reversed by insulin but not fibroblast growth factor

REINITIATION of the growth of quiescent, non-transformed fibroblasts in tissue culture is achieved by addition of serum[1,2] or low concentrations of certain polypeptide hormones[3–6]. These agents which are thought to interact primarily with the surface of the cell[4] presumably require a second signal to be transmitted to the interior. An increase in the intracellular "availability" of low molecular weight nutrients has been suggested to fulfil such a role[7,8]. Mouse fibroblasts placed in different media depleted in certain low molecular weight nutrients reversibly ceased growth in either the G_1/G_0 phase (phosphate, histidine plus glutamine deprivation) or at random (methionine, leucine) in the cell cycle[9,10]. We have found that insulin and other agents stimulated these cells (except those arrested by leucine depletion) to synthesise DNA and divide, whereas fibroblast growth factor (FGF) which normally initiates cell growth in quiescent cultures arrested predominantly by serum depletion of the medium[5,6] was ineffective. This suggests a possible differential basis for their normal mode of action.

Swiss mouse 3T3-4A fibroblasts· in medium containing 10% serum cease growing mainly because of the exhaustion of serum growth factors. Addition of low concentrations (50 ng ml^{-1}) of FGF initiated approximately 40% of these cells to synthesise DNA whereas addition of similar concentrations (50 ng ml^{-1}; Table 1, 250 ng ml^{-1}; not shown) of insulin alone yielded only a small increase. When added with FGF, however, insulin substantially increased the number of cells synthesising DNA, in agreement with previous reports[11,13]. Addition of the original concentration of phosphate (Table 1), glucose or amino acids[14] (not shown) had no effect. This showed that the cultures were completely dependent on the addition of macromolecular components in serum for the reinitiation of cell growth.

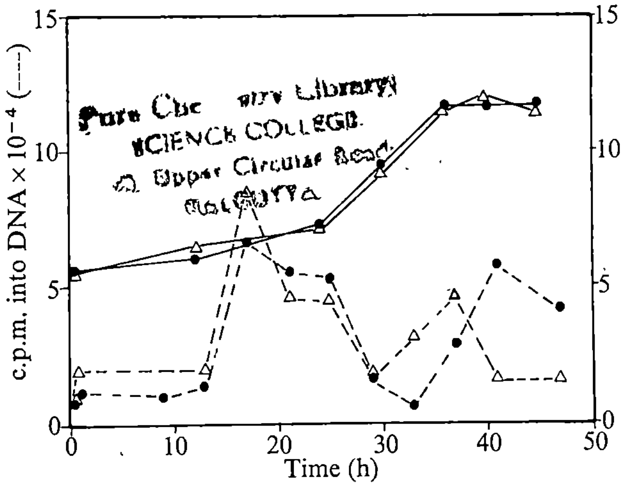

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Fig. 1 Time of induction of DNA synthesis and cell division. Cultures were plated in medium containing 1/100 of the normal phosphate concentration and grown as in Table 1(2). After 3 d insulin (100 ng ml^{-1}) (●) or normal concentrations of phosphate (△) in DEM was added and cultures were exposed for 3 h to ^{3}H-thymidine (3 μCi ml^{-1}) at 3 μM after varying times. The indicated time represents the mid-point of the 3-h exposure to ^{3}H-thymidine (– – –). The c p.m. recorded represent two-thirds of the total radioactivity incorporated into DNA per culture Cell numbers per Petri dish were also recorded (—).

Non-growing 3T3-4A cultures were also obtained by reducing the phosphate, glutamine plus histidine, methionine or leucine concentrations to respectively 1/100, 1/25, 1/25, 1/100 of that present in normal medium. Complete removal of any of these essential nutrients led to cell death within 24 h. Addition of FGF, at 50 ng ml^{-1} (Table 1) or up to 250 ng ml^{-1} (not shown) barely increased the number of radioactively labelled nuclei or the number of cells Addition of hydrocortisone with FGF was also without effect (Table 1). Addition of 250 ng ml^{-1} of insulin alone, however, initiated substantial increases in DNA synthesis (50–60% radioactively labelled nuclei) and in cell numbers in cultures starved for phosphate and histidine plus glutamine (Table 1). Insulin's growth-promoting effect occurred at concentrations from 10 ng ml^{-1} reaching a maximum at about 100 ng ml^{-1} (not shown), while the time required to induce DNA synthesis and cell division was similar to that observed on restoration of the full phosphate concentration

Table 1 Initiation of cell growth in deficient media

Additions	(1) Complete	Missing nutrient (2) Phosphate		(3) Histidine+glutamine		(4) Methionine		(5) Leucine	
	Labelled nuclei (%)	Labelled nuclei (%)	Cell no. ($\times 10^{-5}$)	Labelled nuclei (%)	Cell no. ($\times 10^{-5}$)	Labelled nuclei (%)	Cell no. ($\times 10^{-5}$)	Labelled nuclei (%)	Cell no. ($\times 10^{-5}$)
None	2	2	0.89	6.8	0.14	6	0.22	8	0.22
FGF	39	9	1.08	5.6	0.16	8	0.24	7	0.17
Insulin	10	52	1.57	53	0.26	28	0.27	10	0.19
FGF+I	60	55	1.64	62	0.24	29	0.28	18	0.25
Serum	77	75	1.40	80	0.31	82	0.41	62	0.34
Missing nutrient	2*	82	1.69	62	0.26	89	0.48	81	0.44

Swiss mouse 3T3-4A cells were plated in 2.5 ml of 10% dialysed foetal calf serum and Dulbecco's modified Eagle's medium (DEM) and grown for 12 d (5 d at confluency 0.94×10^5 cells per 3.5 cm dish). (1), As previously described (ref. 6). For the nutrient-depleted cultures 3×10^4 cells were plated into 10% dialysed foetal calf serum and DEM containing 1/100 of phosphate (2), 1/25 of histidine and glutamine (3), 1/25 of methionine (4) and 1/100 of the normal leucine (5) concentrations. After 3 d the cells reached final densities of 1×10^4–8×10^4 per dish. For phosphate and histidine plus glutamine-deprived cultures 93% to 94% of the cells stopped in the G_1 phase of the cell cycle, whereas cultures starved of methionine and leucine mainly ceased growth randomly[10]. Then 50 ng ml^{-1} fibroblast growth factor (FGF), 250 ng ml^{-1} insulin (50 ng ml^{-1} in (1))(I), 20% dialysed foetal calf serum or the normal concentration of phosphate (2), histidine plus glutamine (3), methionine (4), leucine (5) were added. The initial concentration of phosphate in DEM was added to (1) (*). The fraction of cells with radioactively-labelled nuclei and the cell numbers per 3.5-cm dish were recorded as described previously[6]. For autoradiography the cultures were exposed to 3 μCi ml^{-1} ^{3}H-thymidine at 1 μM from 8 to 36 h after additions throughout the entire DNA synthetic period in synchronised cultures. The percentage of cells with radioactively-labelled cell nuclei on addition of 0.5 μg ml^{-1} hydrocortisone (HC) was (1) HC alone 4%; FGF+HC, 40% (2) HC, 8%; FGF+HC, 11%; HC+I, 44%; FGF+HC+I, 60% (3) HC, 5%; FGF+HC, 10%; HC+I, 30%; FGF+HC+I, 70% (4) HC, 7%; FGF+HC, 8%; HC+I, 30% (5) HC, 8%; FGF+HC, 9%; HC+I, 20%. Serum was dialysed for 3 d against 10 volumes of isotonic saline with six changes at 4 °C. It then contained less than 1% of the amino acid concentrations present in the original preparations as determined in the amino acid analyser. In cultures where phosphate or histidine plus glutamine had been completely removed from the medium addition of insulin had no effect.

(Fig. 1). Similar results were obtained with quiescent 3T3-4A cells or human diploid fibroblasts[16] exposed for 3 d or 5 d respectively to 1/100 of the normal glucose or phosphate concentrations. Addition of 250 ng ml^{-1} of insulin doubled the number of cells by 40 h without any increase in the controls (not shown). In 3T3-4A cultures deprived of methionine and leucine, insulin induced only 30% and less than 10% increases in cell numbers and radioactively labelled nuclei respectively (Table 1). Addition of 20% dialysed foetal calf serum or the missing nutrient, initiated the majority (60–90%) of the cells to synthesise DNA and divide in all cultures.

The following control experiments (not shown) suggested that a contaminant[16] was not responsible for insulin's growth-promoting effects. (1) Both 250 ng ml^{-1} of EDTA-dialysed or non-dialysed insulin preparations initiated 30–50% of the phosphate or glutamine plus histidine-starved 3T3–4A cells to synthesise DNA and divide. (2) Extensively boiled insulin or insulin digested with papain bound to beads were completely unable to initiate DNA synthesis and cell division in similar conditions. In the case of other hormones, preparations of ovarian growth factor[17] at 100 ng ml^{-1} increased the incorporation of ^{3}H-thymidine into DNA 10-fold and approximately doubled 3T3 cell numbers when added to quiescent cultures maintained in low phosphate (Table 2) or 1/25 of the normal glutamine (not shown) concentration. Hydrocortisone at 0.5 μg ml^{-1}, oxytocin, vasopressin, glucagon and prolactin (not shown)—all at 50 ng ml^{-1}—had no effect, whereas prostaglandin E_1 at 30 μg ml^{-1} abolished the capacity of insulin to initiate cell division (Table 2).

Insulin may exert its growth-promoting effect by increasing the intracellular transport of the limiting nutrient[7,8] since it predominantly increases uptake rates of the A (sodium-dependent) amino acid transport systems and not the L (sodium-independent) system[18] in various tissues[19]. The A group includes glutamine, histidine, and methionine while the L group includes methionine and leucine[19]. Also EGF and insulin stimulate α-aminoisobutyrate uptake in cultured human fibroblasts[20]. Direct changes in transport rates *per se* cannot solely explain the ability of insulin to initiate cell growth in cultures partially deprived of phosphate, since the same concentrations of FGF and insulin stimulate approximately the same increase in phosphate uptake in medium containing 1/100 of the normal phosphate ion concentration[13].

Here we have shown that insulin, unlike FGF, can initiate DNA synthesis and cell division in fibroblast cell cultures whose growth is completely dependent on the concentration of a number of limiting nutrients. Conversely, FGF initiates substantial increases in DNA synthesis and cell division in cultures allowed to become quiescent without artificial depletion of the culture medium, whereas insulin at the same low concentration has little effect. Thus the hormonal regulation of cell growth in cultures depleted of low molecular weight nutrients differs from that in cultures essentially deprived of serum. This suggests that growth-controlling polypeptide hormones may be divided into at least two distinct classes; those which depend for their action on general changes in certain intracellular nutrient concentrations and those which require additional intracellular signals for their mitogenic activity.

Table 2 Specificity of the initiation of cell growth in phosphate-deficient medium

Additions	^{3}H-DNA (c.p.m. $\times 10^{-5}$)	Cell no. ($\times 10^{-5}$)
None	0.25	0.69
Insulin	2.16	1.12
Oxytocin	0.17	0.82
Vasopressin	0.18	0.95
OGF	2.06	1.24
Hydrocortisone	0.22	0.45
Insulin+PGE$_1$	0.32	0.64

Cells were grown as described in Table 1(2) and after 3 d in medium containing 1/100 of the normal phosphate concentration either 50 ng ml^{-1} of insulin, oxytocin, vasopressin, 100 ng ml^{-1} ovarian growth factor (OGF), 0.5 μg ml^{-1} hydrocortisone, or 30 μg ml^{-1} prostaglandin E_1 (PGE$_1$) were added as indicated. PGE$_1$ was added 10 min before insulin. The c.p.m. of ^{3}H-thymidine incorporated into DNA and the number of cells were recorded as described in Fig. 1 except that the radioactive-labelling time was from 8 to 36 h after additions. Restoration of the normal concentration of phosphate yielded 1.36×10^5 cells.

We thank Dr R. Dulbecco for advice, support and criticism of the manuscript, Dr D Gospodarowicz, Salk Institute for gifts of fibroblast and ovarian growth factors, Dr J. Pike, UpJohn Company for prostaglandin E_1, and Nancy Neyhard for technical assistance. This work was supported by a Jane Coffin Childs Memorial Fellowship to D.K.

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A surface-active agent involved in PVC-induced haemolysis

MANY different forms of PVC powders are manufactured, the variety of the polymers depending on the initial constituents in the reaction mixture. Suspension homopolymers and paste (emulsion or microsuspension) polymers form 91% of the total PVC produced in the US. The latter products range in particle diameter from 0.5 to 30 µm and generally have ionic detergents added during their manufacture, whereas suspension homopolymers have no ionic detergent and are of larger particle diameter (60–180 µm). A preliminary investigation[1] had shown that some forms of PVC particles produced haemolysis *in vitro* and it was suggested that this effect was due to some residual component of the preparation (not vinyl chloride monomer) located on the surface of the particle. The following data support these findings and suggest that the surface-active factor is ionic detergent, used in the manufacture of the paste polymers.

A range of PVC samples was tested for haemolytic activity; fourteen of these were obtained from normal production processes and were used without further treatment; eight samples were specially prepared so that the associated detergent and other factors were different in each case; and a further four samples (20–23) were normal production materials which had been subsequently treated with components (other additives) of the reaction mixture. The results shown in Table 1 are for representative samples of the different types of PVC preparation. Suspension homopolymers have no haemolytic activity unless experimentally treated with detergent (compare samples 19 and 3). A considerable mass of this type of polymer is necessary to produce a significant haemolytic action, and the detergent, its relative concentration and the presence of other additives are also critical factors (compare sample 19 and samples 9 and 8).

All the paste polymers of normal commercial origin or those experimentally produced showed some haemolytic activity although this was low when the detergent code C (a derivative of a sulphated oleate ester) was present (samples 12, 20, 21 and 23). Particularly strong haemolytic potential was found for PVC paste polymers containing code B detergent, sodium dodecyl benzene sulphonate (samples 10, 14 and 18) and in two samples of unknown composition. Paste polymers containing the detergent sodium lauryl sulphate (code A) produced significant haemolysis but were not as active as samples containing detergent B. The haemolytic activity of paste polymers with code A detergent was dependent on: the particle size of the PVC sample (samples 17 and 7 at 10 mg exposure); the total concentration of the detergents associated with the particle surface (samples 15 and 7); and the presence of additives and other detergents (samples 7, 20 and 22).

It has been reported previously[1] that PVC-induced haemolysis could be markedly reduced if the sample was first washed with water or saline and that once removed, the surface-active factor was too dilute or did not retain activity in solution. The present studies have, however, indicated

Table 1 Haemolytic potential of different PVC samples

PVC sample code number	Sample description	Particle size (µm)	Detergent code	% Haemolysis produced by 5 mg	10 mg	20 mg	50 mg
3	SH (NP)	60–120	Absent	—	1	—	1
8	SH (EP)	60–120	B	—	1	—	2
9	SH (EP) low detergent	60–120	A	—	1	—	3
19	SH (EP)	60–120	A†	5	7	13	83
5	Vinylchloride–Vinylacetate copolymer	60–180	Absent	—	0	—	0
7	Paste polymer (NP)	0.5–30	A†	10	99	—	90
15	Paste polymer (EP) low detergent	0.5–30	A†	20	38	100	94
17	Paste polymer (EP, slightly larger particle size distribution)	0.5–30	A†	13	33	99	96
12	Paste polymer (NP)	0.5–30	C	7	10	—	—
20	Paste polymer (as 12 plus other components)	0.5–30	C+A†	7	12	—	—
21	Paste polymer (as 12 plus other components)	0.5–30	C*	5	15	—	—
22	Paste polymer (as 12 plus other components)	0.5–30	C+A	8	81	—	—
23	Paste polymer (as 12 plus other components)	0.5–30	C*	4	7	—	—
10	Paste polymer (NP)	0.5–30	B	100	97	—	—
14	Paste polymer (EP)	0.5–30	B	99	100	—	—
18	Paste polymer (EP) plus sulphosuccinate	0.5–30	B	100	98	—	—
24	Paste polymer (NP)	Unknown	Unknown	99	99	—	—

Haemolysis experiments were carried out as described previously[1] in quadruplicate.

SH, Suspension homopolymer; NP, in normal production; EP, experimentally produced; A, sodium lauryl sulphate; B, sodium dodecyl benzene sulphonate; C, sulphated oleate ester–sodium or potassium; †, at least two additives present; *, single additive present. PVC sample codes are Chemical Industries Association identification numbers.

that some samples (10, 14, 18 and 24) still retain haemolytic potential after washing in buffered saline and that the first wash from 3 of these samples (10, 18 and 24) retains haemolytic potential (45–100%). It is possible that after centrifugation, some very fine particles of PVC remain in the wash material and so account for the haemolytic activity in the wash. Diameter size distribution data suggest that 30–50% of particles in some samples are below 2 μm. The results obtained by further processing the wash material through a 0.2-μm filter before testing for haemolytic potential would support this hypothesis. The wash from one sample (18) still produced 100% haemolysis after passage through the 0.2-μm filter although this effect was reduced to 4% by passage through a 0.01-μm filter. It could also be argued, however, that detergent in the washings binds to the Millipore filter thus reducing the haemolytic potential of the filtered washings.

Most of the paste polymers containing the detergent sodium dodecyl benzene sulphonate (10, 14 and 18) retained high haemolytic activity (75–100%) after washing in saline, an effect particularly pronounced with sample 18 which also contained sulphosuccinate. This effect was not found with PVC samples containing sodium lauryl sulphate (10–20%) which is more readily solubilised. The haemolytic potential of the former samples, however, was totally reduced if they were first treated with ethanol.

Thus, in summary, it is evident that suspension PVC homopolymers have little, if any, haemolytic potential, whereas paste or emulsion powders produce haemolysis probably due to the presence of ionic detergent on their surface. The extent of reaction of these latter PVC polymers is dependent on their particle diameter and size distribution, the concentration and type of detergent and the presence of other processing additives. The results also indicate that certain detergents such as sodium dodecyl benzene sulphonate form a tight association with PVC particles and in this form they are highly reactive membrane-lytic agents. The physiological implications of the above findings are as yet unclear and therefore need assessment by further experimentation.

We thank members of the Chemical Industries Association for assistance in obtaining and identifying the above samples. R.D. thanks the MRC for financial support.

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Received December 9, 1975; accepted January 16, 1976.

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Pioneer neurones in an insect embryo

INSECT sense organs are produced by small groups of specialised epidermal cells[1]. The receptor neurones differentiate at the surface, so developing sensory axons grow inwards from the epidermis to the central nervous system (CNS). How do they find their way? During larval life the axons of newly differentiated sense cells combine with those of neighbouring receptors and thus are guided to the nearest branch of a peripheral nerve which carries them to the CNS[2]. At metamorphosis the axons of adult sensory neurones reach their central destination by growing along persistent larval nerves which are associated with the developing imaginal disks[3–5]. Thus with pathways to the ganglia already established, growth along existing nerves ensures the delivery of each generation of sensory axons to within a few hundred micrometres of their central targets.

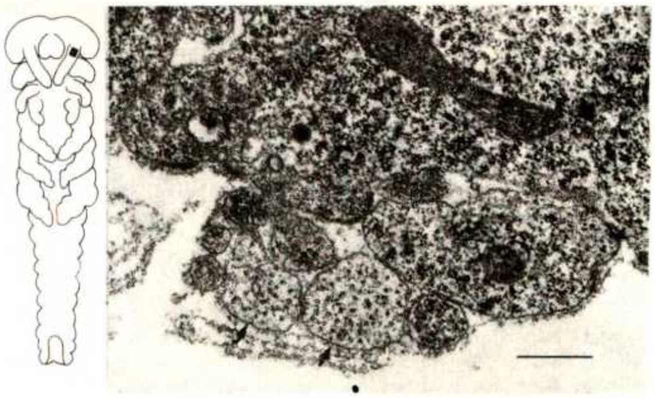

Fig. 1 One of two pairs of axons (arrowed) seen in transverse section through the base of the antenna at the level indicated in the inset. At 105 h of embryonic life at 30 °C. Scale: 1.25 µm.

Just how the connection between the surface and the CNS is first established, whether by an outgrowth of nerves from the centre or by pioneering axons which grow inwards from the surface has never been shown, although some descriptions of embryonic development imply that the first axons to enter the developing appendages are growing outwards from the CNS[6,7]. Presumably these early centrifugal axons would provide a route for the later differentiating sensory fibres to follow in growth to the centre. Here, however, I report observations on the embryonic nervous system of *Locusta migratoria* which show that the first pathways between the epidermis and the central ganglia are formed by axons which grow inwards from peripheral neurones which differentiate early in embryonic life.

Before it rotates round the egg at blastokinesis[8] the locust embryo consists of a segmented band of cells lying on a bed of yolk with its ventral side uppermost. The ganglia develop as a strip on either side of the midline and the base of each appendage is a focus which axons must pass on their way inwards or outwards from the CNS. Timed embryos (30 °C) from a laboratory culture of *Locusta* were processed for electron microscopy[8] and transverse sections were cut through the base of the antennae and the limb buds to look for the early appearance of peripheral nerves.

An initial search revealed that from 110 h of embryonic life onwards, two pairs of axons appear at the base of the antenna (Fig. 1). Where do these axons come from and are they growing into or out of the CNS? Serial sections cut parallel to the long axis of the antenna show that each pair of nerves is produced by a pair of neurones at its distal tip (Fig. 2). From 95 h onwards the two cells of each pair put out processes which grow together to the base of the antenna and enter the embryonic deuterocerebrum. Further series of sections in both planes confirm that there are no other axons in the antenna at this stage, so the two pairs of distal neurones initiate the development of the antennal nerve. During subsequent development the axons of differentiating receptors accumulate about the two sets of pioneering fibres to form two bundles of nerves in the lumen of the antenna. Although the first pairs of axons are naked, the developing bundles become enclosed in the sheathing process of glial cells which appear at intervals along the antenna.

Slightly earlier in development, single pairs of axons appear at the base of each of the three thoracic limb buds. As in the antenna, each pair of nerves is produced by a pair of peripheral neurones. In the limb bud the single pair of cells lies at the anterior margin of the distal end of the lumen (Fig. 2) and from 90 h onwards both of these neurones put out processes which grow inwards to the CNS. Fifteen hours later the two nerves with an expanded growth cone enter the embryonic neuropile from the base of the limb bud (Fig. 3). Further series of sections confirm that these two axons are the first nerves to appear in each of the limb buds, so in the legs as in the antennae, the route from the epidermis to the CNS is pioneered by the ingrowing axons of twin peripheral neurones.

These early differentiating neurones are significant cells in the development of the nervous system. They form the first pathway from the surface to the centre, establish a framework for later innervation and contribute the first peripheral axons to the developing neuropile. During post-embryonic development ingrowing axons which fail to find a peripheral nerve trunk become lost between the epidermis and the basement membrane[2]. The pioneering embryonic fibres reach the central ganglia independently of existing nerves. The axons of the antennal neurones grow directly to its base, but the axons of the limb bud neurones consistently follow a less direct route, which carries them from the anterior half of the distal end of the limb (Fig. 2) over the ventral surface of the leg to the posterior half of its base, where they enter the neuropile (Fig. 3). This indirect route suggests that the path to the CNS is determined not simply by the orientation of the parent neurones but by an interaction between the growing axons and extrinsic cues in the limb bud. The sheathing cells are obvious candidates for a system of signposts. They occur consistently at intervals along the developing appendage and seem to provide a series of stepping stones between the tip of the limb and its base. The last of these cells occurs shortly before the point at which the axons are launched into the developing ganglion (Fig. 3).

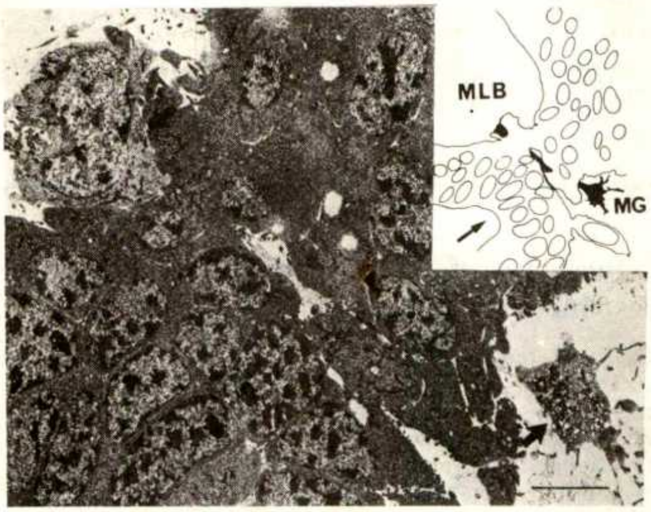

Fig. 3 Inset: camera lucida drawing of 1-μm section of growth cone entering embryonic metathoracic ganglion (MG) from the base of the metathoracic limb bud (MLB) at 105 h of embryonic life. Arrow points anteriorly. Electron micrograph shows part of the same area resectioned for electron microscopy. Notice large sheathing cell (arrowed top left) and expanded growth cone (arrowed bottom right) formed by the terminal expansion of the two axons. Scale: 4 μm.

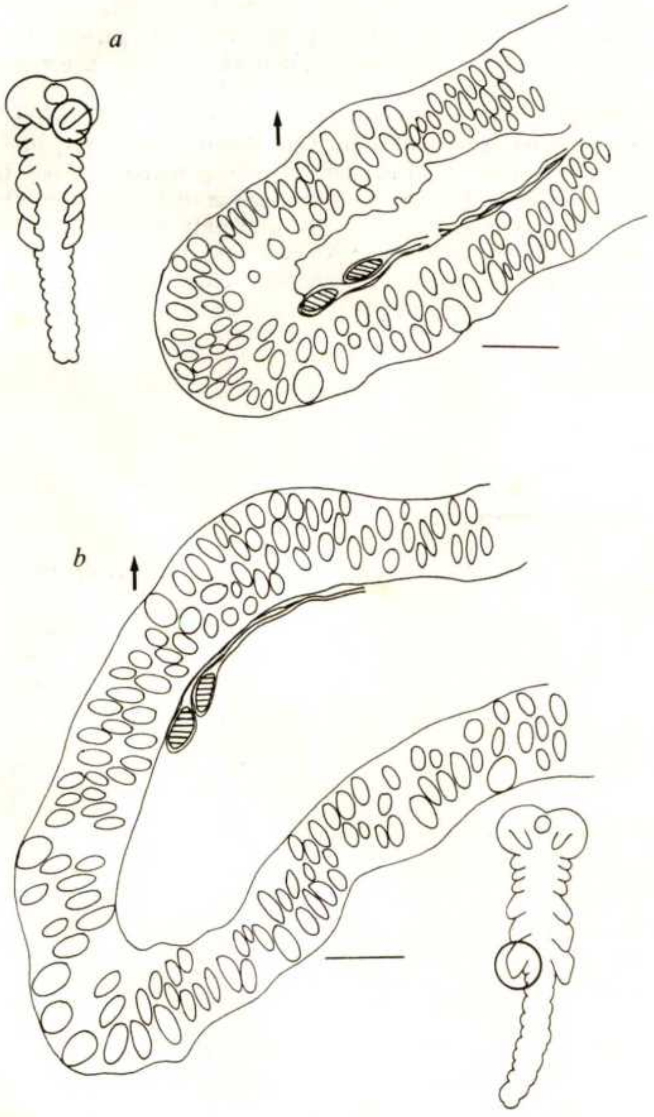

Fig. 2 *a*, One of two pairs of antennal neurones at 100 h of embryonic life. This pair lies on the ventral surface of the antennal lumen, the second pair on the opposite dorsal surface. *b*, Limb-bud neurones from the left metathoracic leg at 95 h of embryonic life. Both camera lucida drawings of 1-μm plastic sections. Arrows point anteriorly. Scales (both): 25 μm.

At the stage at which the peripheral neurones first appear, differentiating central neurones are beginning to put out axons within the ganglia (C.M.B., unpublished) so the pioneer sensory fibres enter the CNS at a crucial stage in its development, when early differentiating ganglion cells lay the foundations of the future neuropile. Perhaps it is fortuitous that similar pairs of cells make the first peripheral pathways in both the antennae and the limb buds. Alternatively an equivalent ground plan may be established in different ganglia by fundamentally similar patterns of growth which reflect the segmental composition of the nervous system. In view of the influence of sensory innervation on the development of insect interneurones[10] and the apparent inductive effect of ingrowing retinal axons on virgin ganglion cells in *Daphnia*[11] it is likely that the early appearance of sensory axons in the embryonic neuropile will be important in determining its structure as well as the distribution of later arriving sensory nerves. This too is now open to experiment.

I thank Nan Cowan for technical assistance and Andrew Jackson for help at the beginning of this work.

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Received November 16, 1975; accepted January 12, 1976.

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Influence of efferent retinal fibres on responsiveness of ganglion cells to light

EFFERENT retinal fibres originating in the nucleus isthmo-opticus[1,2] of the pigeon have been shown, by electron microscopy, to terminate directly on amacrine cells[3]. These findings support the notion of a centrifugal control, originating at the brain, on the retina. Centrifugal fibres exist in several other non-mammalian species[4], but their precise site of termination in the retina has not been determined. In the turtle, stimulation of centrifugal fibres at the level of the optic nerve has a powerful excitatory effect on amacrine cells since it produces a large, "all-or-none" excitatory postsynaptic potential (e.p.s.p.)[5]. Such excitation is in turn transmitted to ganglion cells[5] through the extensive connections between amacrine and ganglion cells[6,7]. Thus ganglion cells are influenced both by the visual input through photoreceptors and other retinal cells, and by a centrifugal input possibly mediated by amacrine cells alone. We describe here some preliminary results concerning the interactions between these two inputs to the ganglion cells.

We used eye-cups of turtles (*Pseudemys scripta elegans*) to which an optic nerve stump 1 cm long was left attached to stimulate both centrifugal fibres and ganglion cell axons. Because there are no reliable criteria to distinguish ganglion from amacrine cells based on their responses to light[5,8], ganglion cells were identified by recording intracellularly their antidromic response to stimulation of optic nerve fibres. A detailed description of the preparation and of the equipment used for intracellular recording is given elsewhere[5].

In more than 50% of ganglion cells, from which intracellular recordings were obtained, illumination of their receptive field (600–800 μm radius) produced photoresponses characterised by a transient depolarisation which did not show any appreciable centre-surround antagonism. These cells were selected for our experiment. Figure 1*a* shows the intracellular responses of a ganglion cell illuminated for 20 ms with a spot (left) and an annulus (right). It seems that the depolarising response maintains approximately the same time course irrespective of the area and pattern of illumination. The antidromic response of the same cell to stimulation of the optic nerve is shown in Fig. 1*b*. Here the spike response (marked by the arrow on the second line) to a single-shock stimulation of the optic nerve (0.5 ms, 2 mA) follows a direct spike produced by injecting outward current through the recording microelectrode. When the interval between the two spikes was reduced progressively, the antidromic response eventually disappeared because it collided with the direct spike travelling orthodromically along the same nerve fibre (Fig. 1*b*, third line). The collision interval found in this cell conforms with the values calculated from conduction times (about 12 ms) and refractoriness (about 8 ms).

When a train of stimuli is applied to the optic nerve, current intensities subthreshold for the antidromic response result in slow, graded, depolarising potentials. Graded responses of ganglion cells to stimulation of the optic nerve with a single shock are usually seen only with very high stimulus intensities (more than twice the threshold for the antidromic response)[5]. The greater efficacy of the low intensity, high frequency nerve stimulation to elicit synaptic

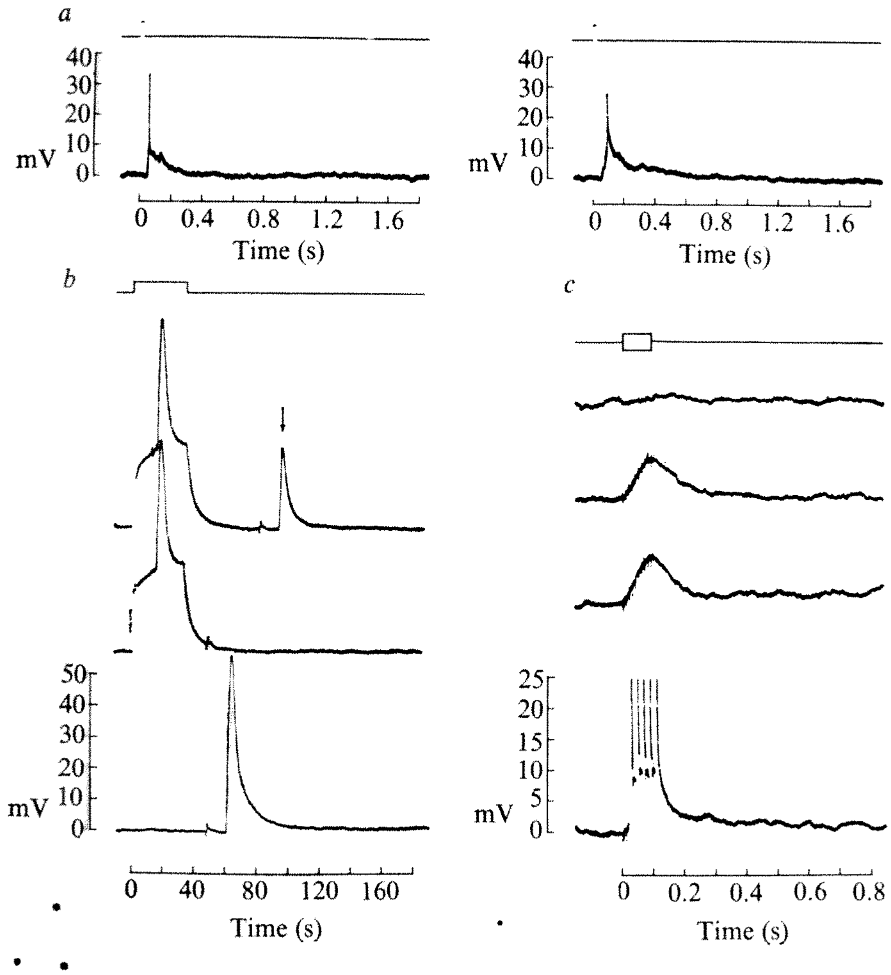

Fig. 1 Intracellular recordings from a ganglion cell in the turtle retina. *a*, Responses to illumination of a circle of 100 μm radius (left) and of an annulus of 180 μm and 1,000 μm inner and outer radius, respectively (right). Zero time coincides with the flash indicated on the first line. *b*, Collision between the antidromic action potential (marked by the arrow on the second line) produced by optic nerve stimulation (0.5 ms, 2 mA) and the direct spike induced by artificially depolarising the cell membrane (third line). The injection of intracellular current is indicated on the first line. In the second line the antidromic action potential fails to invade the cell soma, but is fully developed when not preceded by the direct spike (fourth line). *c*, Ganglion cell responses to repetitive stimulation of the optic nerve (100 ms, 100 per s, 0.1 ms). The stimulus artefact is shown in the first line. The stimulus intensities used, for the responses from top to bottom, are 1.0, 1.5, 1.8 and 2.0 mA, respectively.

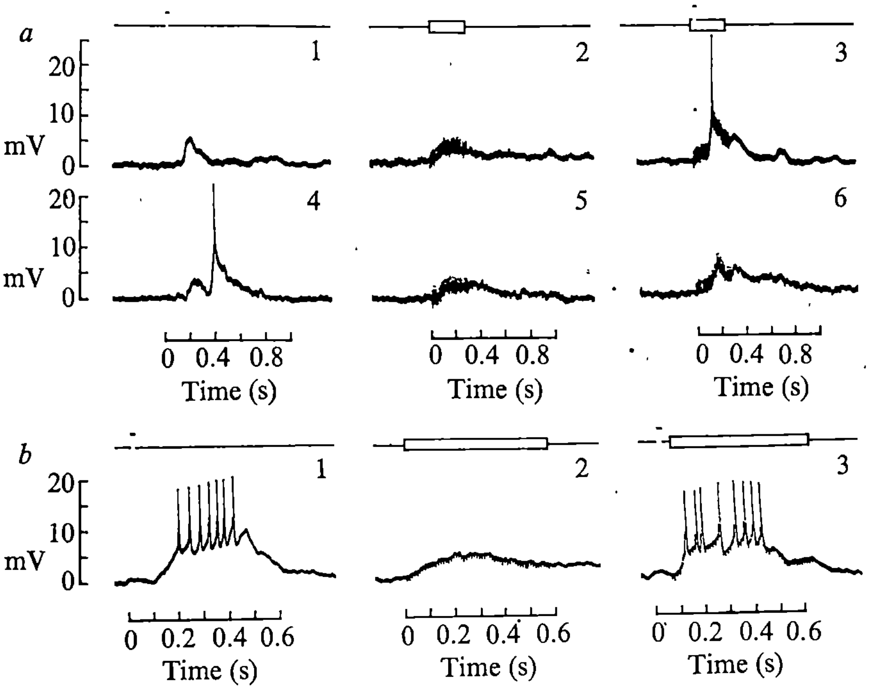

Fig. 2 Interaction between the ganglion cell responses to illumination and to stimulation of efferent fibres. *a*, Same cell as in Fig. 1. *a*1, Slow depolarisation produced by low intensity (4.8 log units of attenuation) illumination of a spot. The light stimulus is indicated in the first line. *a*2, Response to a train of stimuli (300 ms; 100 per s; 0.1 ms; 1.3 mA) applied to the optic nerve (the stimulus artefact is indicated in the first line). *a*3, Ganglion cell response to the combined visual and electrical stimulation. *a*4, Slow potential with a superposed spike produced by low intensity illumination of an annulus (3.6 log units of attenuation). *a*5 and *a*6, as in *a*2 and *a*3. *b*, Recordings obtained from a different ganglion cell. *b*1, Responses to a low intensity (3.6 log units of attenuation) illumination of a spot. *b*2, Response of the same cell to low intensity stimulation of the optic nerve (560 ms; 100 per s; 0.5 ms; 1.4 mA). *b*3, Ganglion cell response to the combined visual and electrical stimulation.

responses is probably due to a process of spatio-temporal summation by which progressively more low-threshold centrifugal fibres are recruited. If the strength of the repetitive stimulation was increased, the threshold for the antidromic response was eventually reached (Fig. 1*c*, fifth line). Thus, two distinct inputs to the same ganglion cell can be distinguished: one originating from photoreceptors excited by light (Fig. 1*a*), the other from activity in the centrifugal fibres (Fig. 1*c*).

The graded depolarisation produced by efferent fibres should affect the responsiveness of ganglion cells to a concomitant photic stimulation because of the resulting change in membrane excitability. Furthermore, since the influence of the centrifugal fibre on ganglion cells is mediated by amacrines[8], ganglion photoresponses may be affected differently according to whether the visual input is conveyed from photoreceptors directly through bipolar cells, or through a more complex pathway involving amacrine cells. These two transmission mechanisms are likely to be activated when the photoresponses are elicited in central or peripheral regions of ganglion receptive fields, respectively[9,10].

Figure 2*a*1 shows a graded depolarising response to a 20-ms illumination of a 100-μm radius spot centred on the recording electrode. High frequency, low intensity stimulation of the optic nerve produced the response shown in Fig. 2*a*2. By combining the two stimuli, the light-induced depolarisation seems to reach the threshold for an action potential (Fig. 2*a*3). On the contrary, a spike obtained by illumination of the periphery of the receptive field (Fig. 2*a*4) would not occur if a graded depolarisation were simultaneously produced by stimulation of the nerve (Fig. 2*a*6). On some occasions the response to the nerve stimulation may, at least partially, add to the annulus-graded photoresponse, but in these instances the centre photoresponse is always greatly enhanced. When centre illumination alone produces a repetitive spike discharge (Fig. 2*b*1), the centrifugal fibre stimulation may decrease the latency of the spike response (from about 200 to 100 ms) and slightly augment the discharge (Fig. 2*b*3). The centrifugal effects are mostly evident on photoresponses to low intensity illumination of either the centre or the periphery: when intensities 1.6–2.4 log units above threshold are used, the photoresponses are modified only slightly.

These results can be interpreted by assuming that a synaptic convergence at the level of the amacrine cells of the centrifugal input and the visual input from the periphery of the receptive field gives rise to an occlusive interaction, which prevents the two depolarising potentials from summating. But summation seems to be possible at the level of the ganglion cell, between the centrifugal input, mediated by amacrine cells and the visual input arising from the centre of the receptive field. This hypothesis may also explain the centrifugal effects on ganglion cell photoresponses observed in the chick[11].

It can be concluded that when a ganglion cell photoresponse coincides with the graded synaptic response to optic nerve stimulation an increased sensitivity to illumination of the central with respect to the peripheral areas is observed. This results in a redistribution of excitability over the ganglion cell receptive field. This effect may be of special functional significance for the detection of light stimuli of low intensity.

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Coupling between rod photoreceptors in a vertebrate retina

The exquisite sensitivity of the scotopic visual system is well known to result, in large part, from a 'pooling' of the signals generated by the rod photoreceptors. It was once assumed that spatial integration of rod signals occurs at cellular sites proximal to the photoreceptors but recent evidence indicates that, in turtle and toad retinae, there is a marked spatial summation of the receptor potential recorded in a single rod[1–3]. In the snapping turtle, *Chelydra serpentina*, we have estimated an individual rod to interact summatively with as many as 200 other rods[3]. Such pooling of signals at the input to the visual system will influence not only the sensitivity of the system but also spatial resolution of visual stimuli.

In the work reported here we demonstrate, by impaling pairs of cells, that the pathway of rod–rod interaction is not through the extracellular medium, nor is the horizontal cell an interneurone in the pathway. The interaction seems to be mediated by specific and direct pathways between rods that involve electrical and/or chemical synapses. The coupling in this pathway is summative and reciprocal. Furthermore, hyperpolarising currents are shown to be more effective than depolarising currents in eliciting responses in neighbouring rods.

We impaled and made simultaneous intracellular recordings from 27 pairs of neighbouring rods. We used an eyecup preparation of the snapping turtle retina[3], into which two micropipette electrodes were advanced independently. This method is similar to that used by Baylor *et al.*[4] on turtle cones. Rods were identified by their response characteristics, verified by intracellular injection of Procion yellow[3]. The separation between the rods of each pair was first determined by finding, for each rod, the positions at which brief flashes of a well focused light spot, 25 μm in diameter, elicited maximal responses. Extrinsic currents were then injected into each rod in turn, and were monitored directly by a current-sensing amplifier; the potential of the neighbouring rod was recorded simultaneously.

The results shown in Fig. 1 are from a typical experiment in which the rods were separated by 75 μm. In Fig. 1*a* the depolarising current (1.8 nA) evoked a depolarisation of about 2 mV in rod 2, whereas the hyperpolarising current (1.5 nA) evoked a hyperpolarisation of almost 9.5 mV. As a monitor of the cell integrity, a light stimulus was presented after each current injection. The slow hyperpolarisation on the right of each voltage record is the normal light response of the rod.

Essentially identical results were obtained when current was injected into rod 2 while monitoring the potential in rod 1. Figure 1*b* shows that depolarising and hyperpolarising current pulses of 1.8 nA, injected into rod 2, elicited in rod 1 a depolarisation of 1.2 mV and a hyperpolarisation of 8.3 mV, respectively.

The control records of Fig. 1*c* were obtained when the electrode was withdrawn from rod 1 to a position nearby in the extracellular medium. They show the resulting intracellular potentials in rod 2 when current pulses of ± 1.6 nA were injected into the extracellular medium near rod 1. In all cases these potentials were less than 1 mV, much smaller than those recorded when both electrodes were intracellular. Similar results were obtained when the current-passing electrode was intracellular and the voltage-recording electrode was just outside the other cell. The small potentials that were still recorded in these conditions must have resulted from current flow in ground loops within the recording system, because they could be recorded even when the current-passing electrode was withdrawn completely from the eye.

Our results confirm that in the snapping turtle, the rod photoreceptors are functionally coupled to one another. More important, several additional points are demonstrated. First, the polarity of the coupled potential in one rod is the same as the potential induced by injecting current into the other rod. This is consistent with previous recordings from single rods, which indicate that the rod–rod interaction is summative. Second, the coupling between rods is reciprocal. Third, the magnitude of the coupled potential depends on the direction of extrinsic current; inward hyperpolarising currents evoked larger coupled potentials than outward depolarising currents of the same strength. Fourth, since the effects of intracellularly injected currents could not be mimicked by injecting similar currents into the extracellular medium, we conclude that summative interactions between rods must be mediated by specific intercellular pathways.

Of the 27 pairs of rods from which recordings were made, 14 pairs were found to be coupled, and in all cases

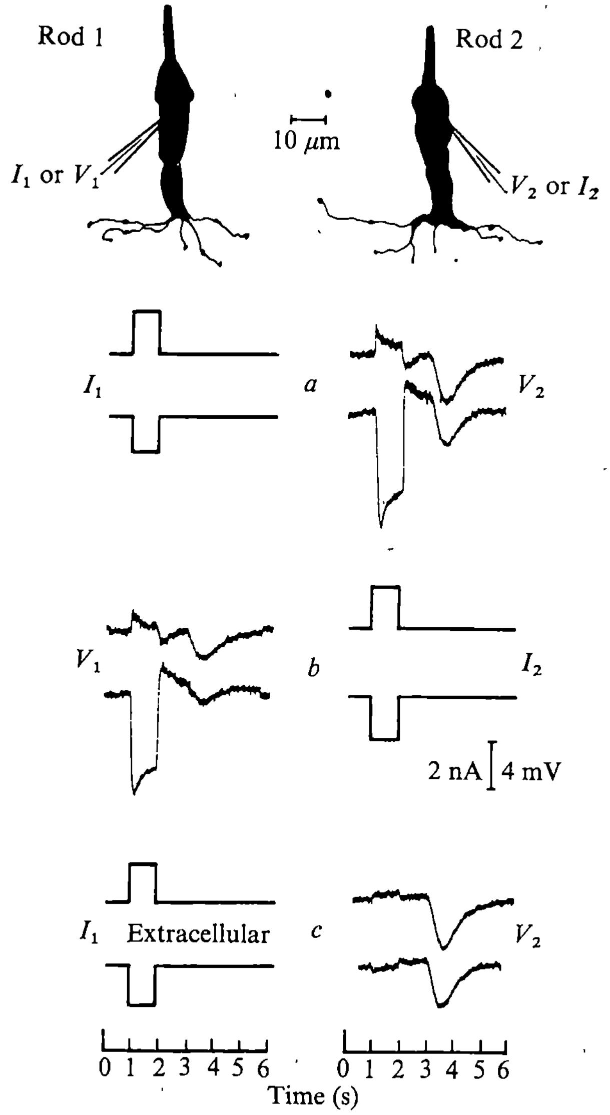

Fig. 1 Simultaneous intracellular recordings from two rods separated by 75 μm. Our recording situation is depicted schematically at the top, using camera lucida drawings of two snapping turtle rods that were delineated by injection of Procion yellow[3]. The current records, I_1 and I_2, show the respective time courses of extrinsic current through rod 1 and rod 2. The potential records, V_2 and V_1, show the respective responses of rod 2 and rod 1, first to a current pulse passed through the other impaled rod and then to a light flash. *a*, Depolarising (outward) current pulse of 1.8 nA and a hyperpolarising (inward) current pulse of 1.5 nA were passed into rod 1. The resulting potential changes are shown in the right column. The top records show the depolarising current in rod 1 and the associated potential change in rod 2; the hyperpolarising current and its effects are shown in the lower records. This convention is also followed in *b* and *c*. *b*, Two 1.8-nA current pulses of opposite direction were passed into rod 2. On the left are shown the potential deflections in rod 1. *c*, As a control, the electrode was withdrawn from rod 1 and current pulses of ±1.6 nA were passed through its tip, while still recording in rod 2. The magnitude and direction of the potentials in all these recordings are given with respect to the resting membrane potential of the rod in darkness.

the coupling characteristics were as described. The maximum separation of coupled rods was about 110 μm. This compares with an upper limit of 150 μm which we found by determining the retinal area over which light responses summate in a single rod[3]. Of the 13 pairs of rods that were not coupled, the rods of 12 pairs were separated by more than 120 μm. For one pair, however, the separation was only 50 μm. In that case the injection of currents into either rod failed to evoke a potential in the other rod, although each rod was clearly influenced by the light responses of rods up to about 150 μm away. Baylor *et al.*[4] showed that the cones of the red-eared turtle, *Pseudemys scripta elegans*, are summatively and reciprocally coupled over distances up to 50 μm. Rods of the snapping turtle exhibit a similar coupling over more than twice that distance.

The only interneurone known to be involved in receptor interactions is the horizontal cell, which has been shown by double impalement recordings to mediate antagonistic interaction between rather widely separated cones in the red-eared turtle[4]. It seems unlikely, therefore, that the horizontal cell would be involved in summative interactions between rods. In addition, the area over which rod signals are summative is much smaller than the receptive field of horizontal cells[3]. In this work we made many simultaneous recordings from a rod and a nearby L-type horizontal cell. When extrinsic currents up to ± 6 nA were injected into the horizontal cell, there was consistently no significant potential evoked in the rod. It thus seems likely that summative rod interaction is mediated by direct pathways between rods.

An interesting feature of snapping turtle rods is that numerous teleodendria, up to 40 μm long, extend laterally across the retina from their broad synaptic terminals. These have been demonstrated by both Golgi staining (Leeper and Stell, personal communication) and Procion yellow injections[3], and are depicted at the top of Fig. 1. The teleodendria may constitute the pathways by which rods can interact selectively with each other.

The teleodendria exhibit both *en passage* and terminal swellings not unlike the presynaptic structures found at chemical synapses in other parts of the nervous system[5]. In our work we could not detect the delay of a chemical synapse, because our electrode resistance of 300–400 MΩ resulted in a recording time constant of 10 ms. Thus the involvement of chemical synapses cannot yet be ruled out.

The greater effectiveness of hyperpolarising currents in evoking potentials in neighbouring rods may not result from the nature of the coupling itself, but from a nonlinear current–voltage relationship in each rod. This is suggested by one experiment in which we impaled a single rod with two electrodes and noted that a hyperpolarising current produced a larger change of membrane potential than a depolarising current of equal strength. Thus further work must be done to elucidate the mechanism underlying the special efficiency of hyperpolarising responses in eliciting responses in neighbouring rods Regardless of its basis, it seems likely that this effect is functionally significant, since the rod's normal light response is a hyperpolarisation.

We thank Dr K. T. Brown for advice and assistance. This work was funded by a postdoctoral fellowship (D R.C.) and a research grant (K.T.B.) from the National Eye Institute.

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Received November 7, 1975; accepted January 5, 1976.

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Diurnal rhythm in rat pineal cyclic nucleotide phosphodiesterase activity

MANY substances in the rat pineal organ exhibit marked diurnal variations, some of these fluctuations are circadian, that is, cued but not driven by environmental lighting. The content of the pineal hormone melatonin[1] for example, is seven- to tenfold higher during the dark phase of the diurnal cycle than during the light phase[2] Similarly, the activities of the melatonin-synthesising enzymes 5-hydroxytryptamine-*N*-acetyl transferase (NAT) and hydroxyindole-*O*-methyl transferase (HIOMT) are also higher in the dark (by factors of 15–70-fold and threefold, respectively[3,4]). The immediate precursor of melatonin, *N*-acetyl 5-HT is 10–30-fold higher in the dark than in the light[5]. In contrast the pineal content of the precursor of *N*-acetyl 5-HT, 5-HT is twofold higher in the light than in the dark phase of the diurnal cycle[6], suggesting that changes in NAT activity regulate changes in melatonin biosynthesis. These changes have been shown to be controlled by environmental light acting through the retina by way of central nervous pathways to the superior cervical ganglion, and thence through postganglionic sympathetic fibres to the pineal body[7]. Noradrenaline released from these fibres acts on β adrenoceptors on the parenchymal pineal cells to stimulate adenylate cyclase, causing an increase in intracellular cyclic AMP which is followed by increased NAT activity and melatonin biosynthesis[8,9] Decentralisation, superior cervical ganglionectomy, administration of β-adrenoceptor blocking drugs or protein synthesis inhibitors can all block the light–dark changes observed in NAT activity in this organ[10,11].

We have recently shown that stimulation of adenylate cyclase by cholera toxin is also an effective stimulus for the induction of NAT in rat pineal organ cultures. The increases in adenylate cyclase activity caused by choleragen also lead to increases in the cyclic AMP hydrolysing enzyme, cyclic nucleotide phosphodiesterase (PDE), so that the increases in cellular cyclic AMP content caused by choleragen are only transient[12].

In view of the importance of PDE in modifying intracellular cyclic AMP levels, and thereby cellular responsiveness to β-adrenoceptor stimulation, we have studied the physiological regulation of this enzyme in a diurnal lighting situation. We report here that PDE activity in the rat

Table 1 Effect of superior cervical ganglionectomy and (–) -propranolol on dark-induced increase in pineal PDE activity

Treatment	Time of death	Cyclic AMP hydrolysis	Cyclic GMP hydrolysis
Sham	1200 light	0.9 ± 0.15	1.9 ± 0.24
Ganglionectomy	1200 light	1.0 ± 0.13	1.9 ± 0.31
Sham	2400 dark	1.6 ± 0.11*	2.9 ± 0.25*
Ganglionectomy	2400 dark	1.0 ± 0.22	2.1 ± 0.43
Saline	1200 light	0.87 ± 0.09	1.7 ± 0.24
(–)-Propranolol	1200 light	0.84 ± 0.05	1.6 ± 0.12
Saline	2400 dark	1.50 ± 0.13*	2.7 ± 0.22*
(–)-Propranolol	2400 dark	0.93 ± 0.10	1.8 ± 0.17

Rats were ganglionectomised bilaterally under Equithesin anaesthesia, or sham operated; allowed to recover for 1 week, and killed at noon and midnight of a 12 h light–12 h dark schedule. Pineals were quickly removed and frozen and PDE activity was determined (see legend to Fig. 1). Other animals were injected with (–)-propranolol (20 mg kg^{-1}) or 0.9% saline subcutaneously at 2000 or 0800 and killed at 2400 and 1200 respectively, the pineals removed and PDE determined Each point is the mean of 5 pineals ± s.e.m. Values are expressed as nmol hydrolysed per 10 min per pineal. *$P < 0.001$ as compared with noon control values.

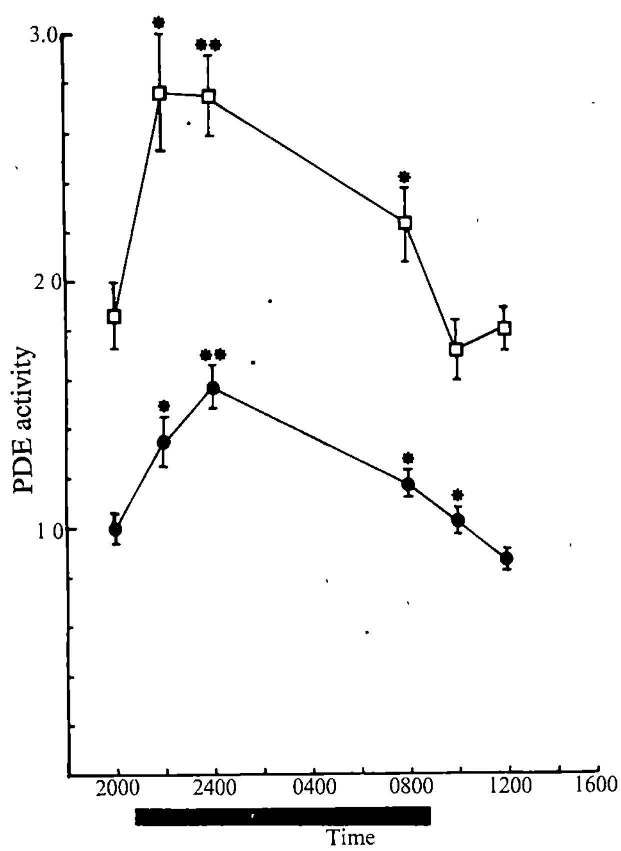

Fig. 1 Diurnal variation in cyclic nucleotide phosphodiesterase in rat pineal organ. Rats were maintained on a 12 h light–12 h dark schedule, with lights on from 0900 to 2100 and were killed at times indicated, and pineals removed and frozen. A single pineal was homogenised in distilled water (100 µl) and PDE activity determined in an aliquot by the method of Thompson and Appleman[13]. Values were corrected for recovery losses of adenosine and guanosine according to the method of Lynch and Cheung[14]. ●, Cyclic AMP hydrolysis at 10^{-6} M substrate concentration; □, cyclic GMP hydrolysis at 10^{-6} M substrate concentration. Each point is the mean of 5 animals. Vertical bars indicate ± s.e.m. Values are expressed as nmol hydrolysed per 10 min per pineal. $^{**}P < 0.001$; $^{*}P < 0.05$ compared with noon values.

pineal is significantly higher during the dark phase of a diurnal lighting regimen, and that this diurnal rhythm seems to be associated with increased β-adrenoceptor stimulation during the dark phase.

Male Sprague–Dawley rats (150–200 g) were housed in groups of 10 in a controlled lighting situation, with lights on from 0900 to 2100; the animals were given food and water *ad libitum* Light was provided by a 60-W incandescent bulb 60 cm from the cage, and temperature was constant at 23–24 °C In some animals, bilateral superior cervical ganglionectomy was performed under Equithesin anaesthesia. Rats were decapitated at various times during the light or dark periods, the heads were chilled on ice and the pineal body quickly removed and stored at −20 °C before assay Cyclic nucleotide phosphodiesterase (PDE) activity was determined by the method of Thompson and Appleman[13] and values were corrected by the method of Lynch and Cheung[14] (see legend to Fig 1).

Table 2 Effect of cycloheximide on dark-induced increase in pineal PDE activity

Treatment	Time of death	Cyclic AMP hydrolysis	Cyclic GMP hydrolysis
Saline	1200 light	0.72 ± 0.05	1.81 ± 0.14
Cycloheximide	1200 light	0.57 ± 0.10	1.6 ± 0.35
Saline	2400 dark	1.25 ± 0.1**	2.6 ± 0.13**
Cycloheximide	2400 dark	0.89 ± 0.04*	2.3 ± 0.06*

Rats were injected at 2000 and 0800 with 30 mg kg^{-1} cycloheximide or 0.9% saline subcutaneously, and killed 4 h later at 2400 and 1200 respectively; pineals removed, and PDE activity determined Each point is the mean of 5 animals ± s.e.m. Values are expressed as nmol hydrolysed per 10 min per pineal $^{**}P < 0.001$ $^{*}P < 0.05$ compared with saline-treated controls killed at noon.

PDE activity in the rat pineal organ exhibited a 24-h rhythm for the hydrolysis of both cyclic AMP and cyclic GMP (Fig. 1), with the highest values occurring in each case in the dark. At 1 µM substrate concentration maximal cyclic AMP hydrolysis in the dark at 2400 was 183% of that at 1200 in the light (Fig. 1), and cyclic GMP hydrolysis at 2400 was 162% of that measured in the light (Fig. 1). Since both cyclic AMP and cyclic GMP are hydrolysed with complex kinetic behaviour in the pineal body (K.P.M. and L.L.I., in preparation), phosphodiesterase activity was also measured at higher concentrations of each nucleotide (100 µM). Both cyclic AMP and cyclic GMP hydrolysis were again maximally increased by 200% in the dark compared to the light values at this substrate concentration.

To determine whether the diurnal rhythm in pineal cyclic nucleotide PDE was controlled by changes in sympathetic nerve activity in the postganglionic fibres innervating the pineal organ, bilateral superior cervical ganglionectomy was performed in some animals. These were killed a week after the operation at midnight and at noon, and pineal PDE measured (Table 1). Ganglionectomy completely blocked the dark-induced increase in pineal PDE, ganglionectomised animals having PDE levels not significantly different from sham-operated controls killed in the light. This suggests that increased sympathetic activity in the dark is responsible for the increased pineal PDE. To determine whether the changes in PDE activity were mediated by noradrenaline release, the β-adrenoceptor blocking agent (−)-propranolol was injected 1 h before the lights went off (2000), or 1 h before the lights went on (0800). PDE activity was again determined at noon and midnight (Table 1) (−)-Propranolol blocked the dark-induced increase in pineal PDE activity, propranolol-treated animals having PDE levels not significantly different from saline-injected controls killed in the light.

Treatment with the protein synthesis inhibitor cycloheximide 1 h before the lights went off (2000), or 1 h before the lights went on (0800) partially suppressed the dark-induced increase in pineal PDE (Table 2). At midnight, PDE levels in the cycloheximide-treated animals were still significantly increased over noon control values, being 125% of the latter for both cyclic AMP and cyclic GMP hydrolysis.

These results are consistent with the recent findings that administration of the β-adrenoceptor agonist isoprenaline *in vivo* can increase PDE activity in the rat pineal organ[16] The diurnal changes observed in PDE activity in the pineal may result from an increased synthesis of PDE enzyme protein in the dark, or possibly through changes in the content of an endogenous activator. In the rat adrenal medulla, an increased concentration of an endogenous activator for PDE has been shown to occur within 20 min after carbamylcholine injection *in vivo*[15], although there is as yet no evidence for the occurrence of such changes in the pineal organ.

Since changes in cyclic AMP seem to be sufficient for the induction of NAT activity in the pineal body[12], and since increases in PDE activity lead to decreases in cyclic AMP content[12], it is clear that alterations in PDE activity can lead to alterations in the sensitivity of pineal biosynthetic enzymes to β-adrenoceptor stimulation, by altering the half life of the intracellular mediator cyclic AMP. The fact that twofold changes in the activity of this enzyme occur in normal physiological conditions is interesting, since diurnal changes in the sensitivity of pineal biosynthetic enzymes to β-adrenoceptor stimulation also occur normally in controlled lighting regimens. Receptor sensitivity is highest during the light period, when PDE activity is lowest, and receptor sensitivity is lowest during the dark period, when PDE activity is increased[17]. Since it seems probable that PDE is induced by high levels of its substrate, cyclic AMP[12], its activity will, therefore, be high after periods of intense receptor stimulation and low after periods of receptor inactivity. The phenomena of sub- and supersensitivity could thus be at least partially explained by a shift in the dose–response curve of adenylate cyclase activation on intracellular cyclic AMP content, controlled by alterations in the rate of degradation of intracellular cyclic AMP. Alterations in ^{3}H-alprenolol binding have recently been observed in sub- and supersensitive pineals[18], suggesting that changes in the population of available receptors may also occur; such changes have also been shown to occur with a marked diurnal cycle[19]. Thus it is probable that several mechanisms contribute to changes in adrenoceptor sensitivity and therefore regulate tissue responses to catecholamine stimulation. For example, superior cervical ganglionectomy causes a striking supersensitivity in the accumulation of cyclic AMP and induction of NAT on β-adrenergic stimulation in daytime rats[20], as well as an increased adenylate cyclase response[21,8]; and our results demonstrate that these phenomena cannot be explained in terms of changes in PDE activity, as the PDE levels are not changed in ganglionectomised animals.

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Received November 26, 1975, accepted January 26, 1976

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In vivo and *in vitro* activation of soluble tryptophan hydroxylase from rat brainstem

TRYPTOPHAN hydroxylation is the rate-limiting step in the biosynthesis of 5-hydroxytryptamine (5-HT) in central serotonergic neurones. In physiological states, tryptophan hydroxylase (EC 1.14.16.4), the key enzyme, is not saturated by its substrate[1]. Thus, three factors which modulate the concentration of tryptophan in brain—the levels of free tryptophan in plasma[2,3], the ratio of total tryptophan levels to those of other neutral amino acids[4], and the uptake process for tryptophan in brain tissues[5,6]—are important in the control of 5-HT synthesis. On the basis of indirect evidence[7,8], it was suggested that tryptophan hydroxylase could also play a regulatory role. Among the mechanisms possibly involved, rapid changes in the enzyme affinity for its substrate and/or its cofactor may take place. Indeed, such changes are responsible for the activation of tyrosine hydroxylase seen in dopaminergic terminals after blockade of dopaminergic receptors by neuroleptics[9]. We used methiothepin(1-[10,11-dihydro-8-(methylthio)-dibenzo(b,f)thiepin-10-yl]-4-methylpiperazine maleate), a potent blocker of central serotonergic receptors[10], to look for a similar activation of tryptophan hydroxylase. Our results indicate that the affinity of this enzyme for its substrate is markedly enhanced in methiothepin-treated tissue.

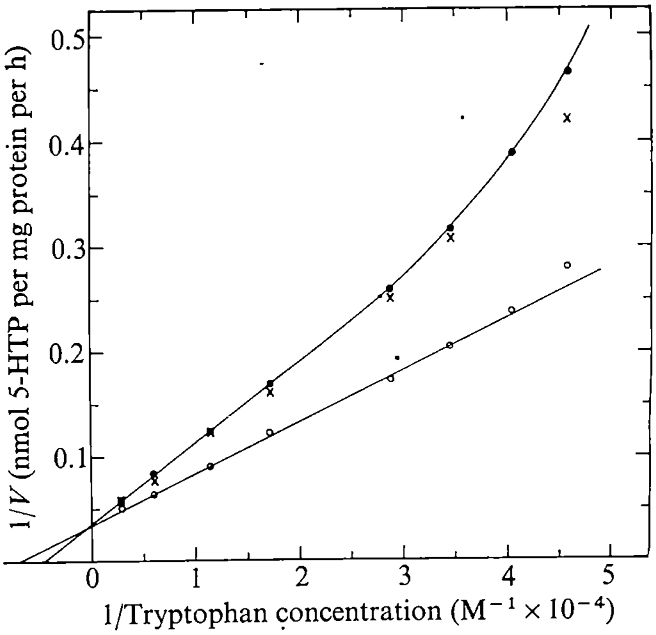

Fig. 1 Double reciprocal plots of tryptophan hydroxylase activity against tryptophan concentration. Effects of *in vivo* methiothepin (○) or methiothepin–LSD treatment (×); ●, control. Methiothepin and LSD were injected as described in Table 2. Soluble enzyme was prepared from the brainstem of rats. Tissues were homogenised (20 s) with a Polytron PT10,OD apparatus in 3 volumes 0.05 M Tris acetate, *p*H 7.6, containing 1 mM β-mercaptoethanol. Homogenates were centrifuged at 30,000*g* for 30 min at 4 °C and the supernatant was used as the final enzyme solution. Tryptophan hydroxylase activity was assayed in the presence of 0.32 mM 6-MPH$_4$ and various concentrations of tryptophan as described previously[12] except that the incubation period was shortened to 15 min. In these conditions, the formation of 5-hydroxytryptophan (5-HTP) was linear with respect to both time of incubation and protein concentration (0.5–1.2 mg ml^{-1}) in the assay mixture. The reaction was stopped by addition of 0.2 ml 4 N perchloric acid Precipitated proteins removed by centrifugation were estimated by the procedure of Lowry *et al.*[13] using bovine serum albumin as standard. 5-HTP was determined in aliquots (0.5 ml) of supernatant by the OPT spectrofluorimetric method[12] or by its native fluorescence in 3 N HCl (ref 1) or 4 N HClO$_4$. Blanks were made by adding 6-MPH$_4$ at the end of the incubation. For the calculations, standards were made by adding known amounts of 5-HTP (1–2 μg) to some samples just before incubation. Statistical calculations were done according to Snedecor and Cochran[14]. Apparent K_m and V_{max} were determined by double reciprocal plotting Each point is the mean of three determinations carried out with tryptophan hydroxylase extracted from groups of 8 rats.

In preliminary experiments, male Sprague–Dawley rats (250–300 g) kept in a controlled environment for 10 d, were injected intraperitoneally with methiothepin (20 mg kg^{-1}) and killed 90 min later by decapitation. Slices of the brainstem were prepared and incubated for 30 min at 37 °C in the presence of ^{3}H-tryptophan. In these conditions (described in detail elsewhere[11]) the rates of formation of ^{3}H-5-HT and ^{3}H-5-hydroxyindole acetic acid (^{3}H-5-HIAA) were markedly stimulated when compared with those in control tissues (+61%). This effect was related partly to an enhanced accumulation of ^{3}H-tryptophan in slices (+30%); however, it was also due to an activation of the rate of tryptophan hydroxylation, as the conversion index

Table 1 Effects of various pharmacological treatments on soluble tryptophan hydroxylase activity from rat brainstem

Treatment	Tryptophan hydroxylase activity
Saline	3.14±0.18
Methiothepin	4.21±0.22*
LSD	3.20±0.12
LSD–methiothepin	2.98±0.24
Apomorphine	3.49±0.15
Apomorphine–methiothepin	4.54±0.28†

Methiothepin (20 mg kg^{-1} intraperitoneally) was injected 140 min before death. LSD (1 mg kg^{-1} intraperitoneally) was injected twice, 160 and 120 min before death. The same schedule was used for apomorphine administration (each 5 mg kg^{-1} intraperitoneally). Tryptophan hydroxylase was assayed as described previously[12] with 0.16 mM 6-MPH$_4$ and 0 24 mM tryptophan. The enzymatic activity is expressed in nmol 5-HTP formed per mg protein and per h. Each value is the mean ± s.e.m. of determinations made on groups of 8 rats.

*$P < 0.05$ compared with saline-treated rats.

†$P < 0.05$ compared with saline- or apomorphine-treated rats.

of tryptophan into 5-hydroxyindoles (^{3}H-5-hydroxyindoles/tryptophan specific activity) was 33% higher in slices of treated rats. A similar, but less pronounced stimulatory effect (+24%) of methiothepin on *in vitro* 5-HT synthesis was observed when the drug (30 μM) was added directly to the incubating medium. This could be prevented when slices were incubated in the presence of both methiothepin (30 μM) and LSD (10 μM, D-lysergic acid diethylamide bitartrate, Sandoz) a well known agonist of 5-HT receptors.

When estimated in the presence of subsaturating concentrations of tryptophan (0.24 mM) and cofactor (0.16 mM) the activity of tryptophan hydroxylase isolated from tissues of methiothepin-pretreated rats (20 mg kg^{-1} intraperitoneally) was increased by 30–40% (Table 1). This stimulation was observed from 90 min to 5 h after administration of the 5-HT blocking agent, whereas methiothepin incubated directly with the control enzyme was ineffective and inhibited the enzyme activity at high concentrations (−27% at 50 μM). LSD injected twice (each 1 mg kg^{-1} intraperitoneally) 20 min before and 20 min after methiothepin (20 mg kg^{-1} intraperitoneally) prevented the stimulation of tryptophan hydroxylase estimated 140 min after the 5-HT antagonist injection (Table 1). The potent agonist of dopaminergic receptors, apomorphine (5 mg kg^{-1} intraperitoneally, Hoffmann–La Roche) injected 20 min before and 20 min after methiothepin did not alter the activation of tryptophan hydroxylase induced by the 5-HT blocking agent (Table 1). In the presence of a high concentration of tryptophan (0.5 mM), control tryptophan hydroxylase exhibited an apparent K_m for the pteridine cofactor, 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine. (6-MPH$_4$ Calbiochem), equal to 183 μM. The enzyme isolated from the brainstem of methiothepin-treated rats exhibited the same affinity for the cofactor and its V_{max} was slightly but not significantly increased (Table 2). Conversely, methiothepin treatment increased significantly the affinity of the enzyme for tryptophan (Table 2) without changing its V_{max}. The determination of these kinetic parameters was carried out with a subsaturating concentration of 6-MPH$_4$ (0.32 mM) as the reduced pteridinic cofactor inhibited tryptophan hydroxylase activity when its concentration reached 0.4 mM (M.H. and S.B., unpublished). After double reciprocal plotting, the curve obtained with the enzyme extracted from control tissues was parabolic with an upward concavity suggesting a cooperative effect (Fig. 1); (Hill plot gave an n value close to 2). In contrast, the curve became linear for the enzyme of methiothepin-treated rats. As shown in Fig. 1, when rats were treated as in the previous experiment both with methiothepin and LSD, the affinity of the enzyme for tryptophan returned to the control value.

The change in affinity of the enzyme for its substrate in methiothepin-treated rats as well as the parabolic curve observed for the enzyme of control tissues (Fig. 1) suggest that tryptophan hydroxylase shares allosteric properties with other polymeric enzymes. Such enzymes can be activated by mild trypsinisation or exposure to small concentrations of detergents. Therefore, the effects of trypsin (1 : 250, Difco) and sodium dodecyl sulphate (SDS, Sigma) on the kinetic characteristics of the enzyme were examined. A tryptophan hydroxylase solution was incubated at 30 °C for 30 min in the presence of trypsin (6.5 μg proteolytic enzyme per mg protein in the brainstem supernatant). The reaction was stopped with 500 μg soybean inhibitor (Worthington) and the enzyme activity assayed as in Fig. 1. In these conditions, the affinity of tryptophan hydroxylase for tryptophan was markedly increased (Table 2). The V_{max} was reduced but a similar change occurred when the enzyme was preincubated for 30 min at 30 °C in the absence of trypsin, indicating that this alteration was simply related to the instability of tryptophan hydroxylase. When the enzyme assay was carried out in the presence of 0.01% detergent, a significant change in the enyzme affinity for its substrate was also seen. Both with trypsin and SDS, the affinity of tryptophan hydroxylase for the cofactor as well as its V_{max} (in the presence of 0.5 mM tryptophan) were significantly increased (Table 2).

The changes in 5-HT synthesis induced by the 5-HT antagonist, methiothepin, may be related to an increased transport of tryptophan in tissues, but also to an activation of tryptophan hydroxylase. At it could be directly observed *in vitro*, the affinity of the enzyme for its substrate amino acid was selectively

Table 2 Effects of various treatments on kinetic parameters of soluble tryptophan hydroxylase from rat brainstem

	Tryptophan		6-MPH$_4$	
	K_m(μM)	V_{max}	K_m(μM)	V_{max}
Control	234±17 (11)	22.1±1.4	183±15 (8)	23.7±1.6
Methiothepin	139±16* (5)	23.1±2.4	181±17 (5)	26.1±0.8
SDS	110± 5* (5)	27.0±3.0	102±11* (5)	46.4±5.2*
Preincubated control	213±22 (6)	11.2±0.8	166±14 (5)	12.2±0.9
Trypsin	106± 6* (6)	12.3±1.1	104±12* (4)	19.1±1.5*

Determinations of apparent K_m and V_{max} of tryptophan hydroxylase with tryptophan concentrations in the range 20 μM–0 5 mM were carried out in the presence of 0.32 mM 6-MPH$_4$. When the concentration of 6-MPH$_4$ was the variable, tryptophan concentration was maintained at 0.5 mM. *In vivo* treatment with methiothepin (20 mg kg^{-1} intraperitoneally) occurred 120 min before death. SDS (0.01%) was added to the standard assay mixture without preincubation. The effect of trypsin was analysed after 30-min preincubation of tryptophan hydroxylase solution with the proteolytic enzyme (6.5 μg trypsin per mg protein of the brainstem supernatant) at 30 °C. 'Preincubated control' corresponds to tryptophan hydroxylase solution incubated for 30 min at 30 °C in the absence of trypsin. V_{max} is expressed in nmol 5-HTP formed per mg protein and per h. Each value is the mean ± s.e.m. of determinations made in 4–11 experiments (numbers of experiments indicated in parentheses).

*$P < 0.05$ compared with respective control values.

two proteins present in active enzyme prepared from human foetal and monkey livers. This protein was also demonstrated in livers obtained from the patients who died of unrelated diseases (whose livers were normal). Study of this protein, and of fragments of the native enzyme which seem to copurify, may be useful in the elucidation of the defect in PKU. The general approach may be useful in the study of other inborn errors of metabolism in which the enzyme involved is rapidly inactivated after death or when the mutant enzyme is inactive

Monkey liver phenylalanine hydroxylase was purified as already described[4]. The small modifications (Fig. 1) which were made for crude extracts of human livers did not alter the proteins isolated The specific activity of each preparation is shown in Fig. 1. The proteins thus purified were then studied by the Gradipore technique[6] modified by the use of sodium dodecyl sulphate (SDS) (Fig. 1).

Purified monkey liver enzyme yielded two proteins of similar molecular weight (Fig. 1*b* and *g*) which ran between the two marker proteins which were used (catalase molecular weight 57,500 and ovalbumin molecular weight 45,000) (Fig. 1*a*). This indicates molecular weights very similar to those found when enzyme purified from rat liver by traditional methods was analysed by SDS electrophoresis—two proteins with molecular weight of 50,000 and 49,000 respectively have been described[7].

The preparation from the 16-week foetus showed a similar pair of proteins (Fig. 1*c*). The preparation from the 34-week foetus (Fig. 1*f*) from the normal human liver (Fig. 1*e*) and from the PKU liver (Fig 1*d*) all showed only the protein of smaller molecular weight (protein 2). Additional bands corresponding to smaller protein species were also noted in the preparations from PKU liver, from normal livers, and from the 34-week foetal liver (Fig. 1*d–f*).

Phenylalanine hydroxylase should be the only protein in liver which can bind to the pteridine adsorbent at the high concentration of NaCl (0.8 M) used and specifically eluted by phenylalanine. Consequently there is good reason to believe that the protein purified by this technique represents phenylalanine hydroxylase in an active or inactive form. Material isolated from monkey liver and from the foetal livers showed substantial phenylalanine hydroxylase activity. One protein species (protein 2) purified from the normal human livers and from the PKU liver behaved in the same way as one of the proteins obtained from those livers with enzyme activity, and we believe that this protein represents a component of phenylalanine hydroxylase. This is supported by the close correspondence between the behaviour of the monkey liver enzyme purified by this method and that described for purified rat liver phenylalaline hydroxylase[7].

Kaufman[7] has produced smaller species which are still active, by proteolytic cleavage of purified rat liver phenylalanine hydroxylase. This type of molecule may be represented among the smaller species purified from human livers (Fig. 1*d–f*).

Protein 1 was found only in the active preparations from monkey liver and from the 16-week foetus. Rapid degradation after death may explain the absence of this protein from the 34-week foetal liver, and from livers obtained at autopsy from older patients which had low activity. The degradation of this protein species may explain the rapid loss in enzyme activity observed after death[3]. The same mechanism may explain the absence of this band from the PKU liver preparation It is also possible, however, that it represents the effect of the PKU mutation

The next step must be to study the strucure of the proteins isolated from this PKU liver. The preparations analysed in the experiment illustrated in Fig. 1 have been subjected to isoelectric focusing. But because of the considerable heterogeneity revealed, it was not possible to detect a simple charge-change mutation.

We thank Phillip Grattan and Ian Jennings for technical assistance and Drs K. Hayes, D Pitt and D. Sillence and M. A Winther and the Departments of Pathology of St Vincent's Hospital and the Royal Children's Hospital for liver samples. We thank Dr B. Davidson for criticism of the manuscript. This work was supported by an NH and MRC grant and R.G.H.C. was the recipient of a Queen Elizabeth II fellowship.

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Received November 14, 1975; accepted January 14, 1976.

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Inhibition of reverse transcriptases of primate type C viruses by 7S immunoglobulin from patients with leukaemia

WE have characterised[1] type C virions which are released spontaneously from a diploid strain of human embryonic fibroblasts. These 'HEL-12 virions' have structural and biochemical properties of known RNA tumour viruses and are antigenically related to subhuman primate type C viruses. Immunological analyses have so far excluded cross reactivity between HEL-12 virions and avian (ASV), murine MuSV, or feline RNA sarcoma or leukaemia viruses.

Several lines of evidence strongly suggest that HEL-12 virions originate in the human cell strain from which they were isolated[1]. Unambiguous proof of their human origin is, however, difficult to obtain, particularly in the absence of an undisputed human type C reference virus. We have approached this problem by asking if humans have humoral immunity to HEL-12 virion antigens. Because antibody to RNA-dependent DNA polymerase (reverse transcriptase[2]) is easily quantifiable and can be used to distinguish primate from non-primate type C viruses[3], purified immunoglobulin (Ig) from eight patients with various types of leukaemia was assayed for anti-reverse transcriptase antibody. We found that the 7S Ig from the plasma of two leukaemic patients significantly inhibited the incorporation of ^{3}H-dTTP by the reverse transcriptase of HEL-12 virions, woolly monkey fibrosarcoma (simian sarcoma) virus (SiSV)[4] and baboon endogenous virus (BEV)[5].

Ig was purified from the plasma of eight patients with leukaemia by differential fractionation with ammonium sulphate and rate zonal sedimentation of the precipitated Ig in 10–40% linear sucrose gradients[6]. The major protein peak migrated with a sedimentation coefficient of 7S (Fig. 1). The gradient profiles of Ig from leukaemic patients were not substantially different from those obtained with Ig from normal adult volunteers (Fig. 1). The pooled, purified 7S fraction from each patient was dialysed exhaustively, and antibody was quantitated by radial immunodiffusion in Agarose plates embedded with rabbit anti-human IgG, IgA or IgM (ref. 8). All precipitated protein was accounted for as either IgG (~90%) or IgA (~10%); no IgM was detected. Electrophoresis in sodium dodecyl sulphate (SDS)-polyacrylamide gels[9] revealed that

Table 1 Inhibition of type C virus reverse transcriptases by purified 7S Igs from eight leukaemic patients

Patient	Haematological diagnosis	Maximal Ig concentration (μg)	Maximal inhibition (%) of reverse transcriptases HEL-12	HL32V-1	BEV	SiSV	G-MuLV	KiMuSV (KiMuLV)	SR-RSV
N.K.	ALL	56	<10	<10	<10	<10	<10	13	<10
J.J.	ALL	60	<10	12	13	<10	<10	<10	<10
D.W.	CML	63	<10	ND	<10	<10	<10	<10	<10
E.P.	AML	76	<10	<10	<10	<10	<10	ND	12
T.K.	AML	80	<10	<10	12	<10	<10	<10	<10
B.J.	AML	72	<10	12	<10	<10	<10	<10	<10
M.R.	AML	64	53	11	72	42	18	<10	<10
R.M.	AML	70	66	37	81	32	<10	12	<10

Ig was purified from plasma of each patient as described in Fig. 1 Growth and purification of HEL-12 virions, G-MuLV, and Kirsten murine sarcoma (leukaemia) virus (KiMuSV (KiMuLV)) described previously[1,9] BEV, gift of Dr R C Gallo, National Cancer Institute, Bethesda, Maryland. HL23V-1, type C particle originally isolated by Gallagher and Gallo[11] now grown in dog thymus cells[12] SiSV and Schmidt–Ruppin strain of Rous sarcoma virus (SR-RSV), (Electro-Nucleonics Laboratories, Bethesda, Maryland) were disrupted and frozen immediately on receipt[1]. To test for Ig inhibitory activity, all viruses were disrupted for 5 min at 37 °C in 0 1 % Nonidet P40 (NP40) detergent, 5 μl virus (~ 12,000–80,000 c.p.m. ^{3}H-dTMP rendered acid precipitable in 40 min using poly(A)·oligo(dT)$_{12-18}$ as template) were mixed with 5 μl 0 6 M KCl solution containing 50 mg ml^{-1} bovine serum albumin (KCl–BSA) To this was added 20 μl dilution of appropriate Ig. Entire mixture incubated for 2 h at 4 °C; 170 μl reverse transcriptase reagents (0.025 % NP40, 25 mM Tris-HCl, *p*H 8 1, 7m M 2-mercaptoethanol, 0 2 mM MnCl$_2$, 60 mM KCl, 1 μg poly(A)·oligo(dT)$_{12-18}$ (Collaborative Research, Waltham, Massachusetts) 5 μCi ^{3}H-dTTP (specific activity 55 Ci mmol^{-1}, Schwarz–Mann, Orangeburg, New York) and 0 0083 mM 'cold' dTTP (Sigma) were added; entire mixture incubated at 37 °C for 40 min. Reaction mixture chilled quickly in ice and precipitated by addition of 25 volumes ice-cold 5 % TCA–2 % sodium pyrophosphate Acid-precipitable radioactivity determined[9,13]. Inhibition expressed relative to reactions containing virus without Ig. This reference value determined from average of four identical reaction mixtures. Figures represent percentage inhibition of reverse transcriptase at highest Ig concentration used. In cases in which inhibition was observed, value given represents a plateau level, at which increasing the Ig concentration resulted in no further inhibition of enzymatic activity

ALL, Acute lymphocytic leukaemia; CML, chronic myelocytic leukaemia; AML, acute myelocytic leukaemia; ND, not determined

more than 95% of the purified protein migrated, as expected for heavy and light chains of IgG and IgA (ref 10).

The extent of inhibition of the reverse transcriptase from various type C viruses was compared using purified 7S Ig from each patient (Table 1). Ig from six of the patients did not inhibit significantly the reverse transcriptase of any of the viruses tested. 7S Ig from patients M.R. and R.M., however, reduced the incorporation of ^{3}H-dTTP by reverse transcriptase in the following order: BEV>HEL-12> SiSV>HL23V-1 (ASV7573), (Table 1 and Fig. 2). These findings were confirmed repeatedly for Ig fractions obtained at different times from each of the patients. The difference in inhibition between the enzymes from HEL-12 virions and SiSV is probably related to the antigenic heterogeneity of the former[14]. We have found[14] that the HEL-12 virion population contains at least two components, one with antigenic characteristics of SiSV, the other with properties of BEV. Interestingly enough, the reverse transcriptase of a type C virus isolated by Gallagher and Gallo[11,12] and referred to as HL23V-1, was not significantly inhibited by 7S Ig from patient M.R. The reasons for the lack of reverse transcriptase inhibition of this isolate are unclear, even though the HL23V-1 virus is known to be related to SiSV. The results may be attributable to qualitative differences between the 7S Ig fractions of patients M.R and R M., as well as antigenic differences between the reverse transcriptases of HEL-12, HL23V-1 and primate viruses such as SiSV. We have also examined for inhibitory activity the 7S Ig fraction of six normal volunteers. The viruses used as sources of reverse transcriptase included HEL-12, SiSV,

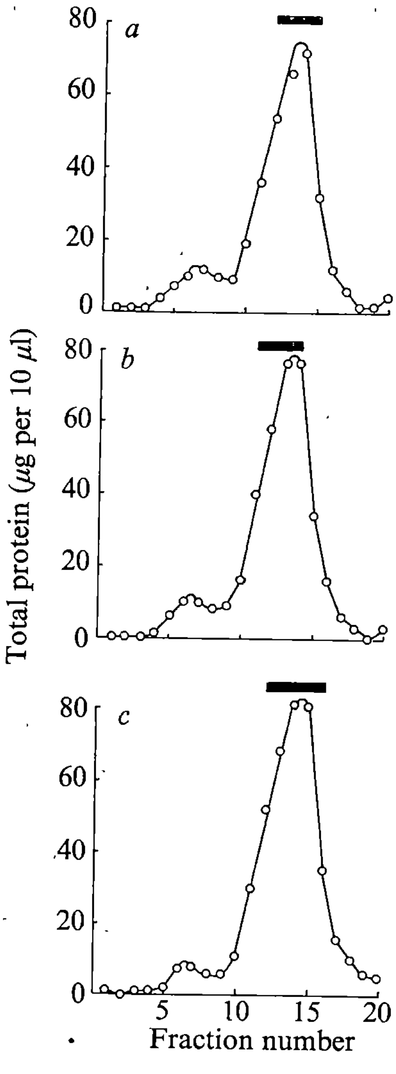

Fig. 1 Representative zonal sedimentation profiles of Ig from patients M.R. (*a*) and R.M. (*b*), and a normal volunteer (*c*). Blood from patients drawn into sterile, heparinised tubes, allowed to stand at 4 °C for 3–8 h and centrifuged at 1,100 r.p m for 10 min. Plasma stored at −20 °C. For Ig purification, 1.0 ml plasma from each patient was diluted with an equal volume of TNE buffer and slowly mixed with 2.0 ml saturated solution ammonium sulphate at 4 °C. After stirring for 30 min, precipitate centrifuged for 30 min at 2,000*g*. Supernatant fluid discarded, pellet resuspended in 2.0 ml TNE buffer, and precipitation by ammonium sulphate repeated. After centrifugation at 2,000*g*, pellet resuspended in 300 μl TNE buffer and dialysed exhaustively at 4 °C against 0.04 M phosphate buffer, *p*H 6.9 300 μl dialysate layered on 5 ml linear 10–40 % sucrose gradient in TNE buffer and centrifuged at 4 °C in a SW50.1 rotor at 35,000 r p.m. for 16.25 h 20 equal-volume fractions collected from bottom of tube, and 10 μl aliquots assayed for protein according to method of Lowry *et al.*[7]. IgG purified on Sephadex G-200, included in a parallel gradient, sedimented at fraction 14. Fractions indicated by bars were pooled, dialysed exhaustively against 0.1 M Tris-HCl, *p*H 8.0, and stored at −20 °C. Direction of sedimentation from right to left

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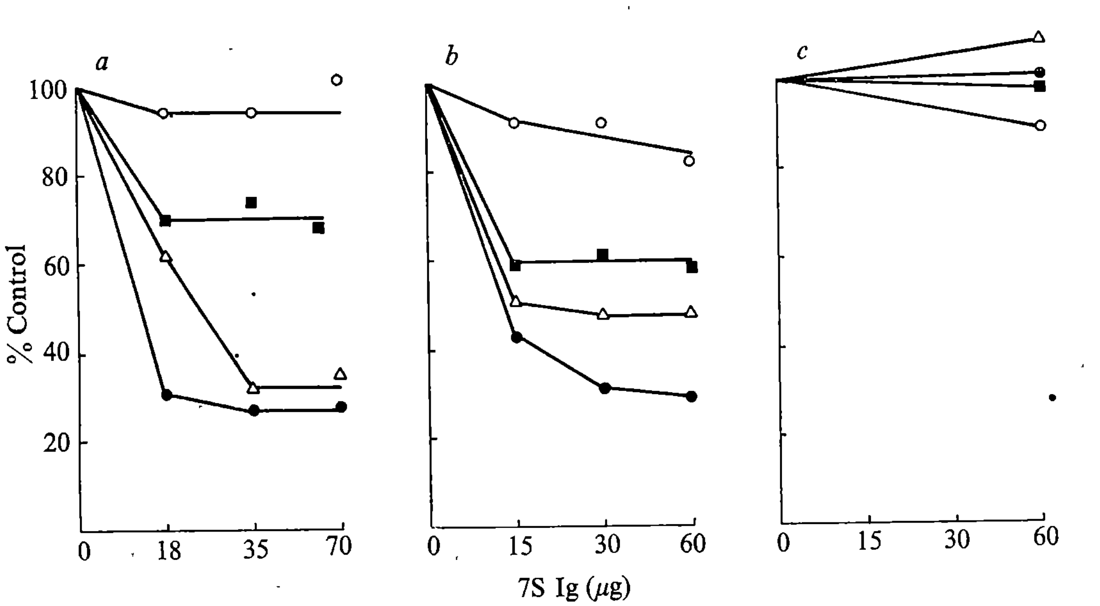

Fig. 2 Inhibition of type C virus reverse transcriptases by purified 7S Ig. 20 μl dialysed 7S Ig fraction from patient M R (*a*) or R.M. (*b*) or from a normal volunteer (*c*) incubated for 2 h at 4 °C with 5 μl KCl–BSA and 5 μl appropriate NP40-disrupted type C virus (12,000–80,000 c.p m ^{3}H-dTTP incorporated in 40 min). Reverse transcriptase reagents added (170 μl). Acid-precipitable ^{3}H-radioactivity measured after 40 min incubation at 37 °C All values expressed relative to average of quadruplicate reactions for each virus run in absence of 7S Ig. Each point represents average of duplicate samples. ○, G-MuLV; ■, SiSV; △, HEL-12 virions; ●, BEV

and Gross murine leukaemia virus (G-MuLV). In none of the cases was a greater than 10% inhibition observed at comparable 7S Ig concentrations (not shown).

The above observations with the 7S Ig of patients M R. and R.M. were extended to the purified reverse transcriptases of avian myeloblastosis virus (AMV) and SiSV. At the concentrations tested, 7S Ig from patients M.R. and R.M did not significantly inhibit the activity of AMV reverse transcriptase (Fig. 3). Substantial inhibition of the SiSV enzyme was, however, observed in the case of both Igs.

Since destruction of the RNA template or of the enzymatic poly(dT) product by plasma nucleases could account for the observed inhibition, 7S Igs were incubated with ^{3}H-poly(A) or with poly(A)·^{3}H-poly(dT) (Table 2). As determined by hydrolysis of the labelled polynucleotides, Ig from leukaemic patients did not contain poly(A) nuclease activity nor was ^{3}H-poly(dT) hydrolysed to a significant degree by any of the Igs tested.

The inhibition might also have been due to the presence of anti-nucleic acid antibodies in the Ig fraction. Aliquots of Ig were therefore incubated with ^{3}H-poly(A) or with poly(A)·^{3}H-poly(dT). The Igs were precipitated with half saturated ammonium sulphate which precipitates only nucleic acid molecules complexed with antibody[17]. As shown in Table 2, anti-nucleic acid antibodies were not detected by this assay method in any of the samples tested.

Why do patients with leukaemia develop a humoral antibody against an internal virion component such as reverse transcriptase? Three explanations can be offered: leukaemic leukocytes may produce type C viruses which are degraded and release antigenic reverse transcriptase[11,18,19] related to the reverse transcriptase of HEL-12 virions; lysis of leukaemic leukocytes may release "particle-associated reverse transcriptase[20–22]" (note that both patients with inhibitory antibody had undergone long term chemotherapy and had survived for approximately one year from the time of initial diagnosis; however, the patients were never in haematological remission); reverse transcriptase may be exposed at the surface of leukaemic leukocytes, as are other internal virion proteins in infected animal cells[23–25].

Irrespective of the mechanism by which reverse transcriptase exerts its antigenic effect, the antibody response observed in the two patients seems highly specific for the reverse transcriptases from viruses of primate origin. The specificity of reverse transcriptase inhibition was substantiated using purified enzyme preparations derived from stocks of AMV and SiSV. Thus, it cannot be argued that

Table 2 Nucleases and anti-nucleic acid antibodies in 7S Ig from leukaemic patients

Patient	7S Ig (μg)	Nuclease activity*, % hydrolysis of		Anti-nucleic acid antibody†, % precipitated	
		^{3}H-poly(A)	poly(A)·^{3}H-poly(dT)	^{3}H-poly(A)	poly(A) ^{3}H-poly(dT)
N.K.	28	13.1	4.0	<0.1	11.2
J.J.	30	−6.4	−13.0	0.1	7.9
D.W.	32	20.0	−10.7	<0.1	7.2
E.P.	38	−2.8	5.4	0.1	6.3
T.K.	40	7.6	4.5	0.1	5.8
B.J.	36	−8.2	−15.1	<0.1	12.8
M.R.	32	1.5	4.2	<0.1	13.5
R.M.	35	−10.9	−4.4	<0.1	5.8

^{3}H-poly(A), Miles Laboratories, Elkhart, Indiana. Poly(A)·^{3}H-poly(dT), synthesised in a standard reverse transcriptase reaction[1,9,13] using purified reverse transcriptase from AMV as enzyme source (Schwarz–Mann). Reaction mixture diluted tenfold in TNE buffer (0.1 M NaCl, 10 mM Tris-HCl, *p*H 7.3, 1 mM EDTA) and extracted once with TNE-saturated recrystallised phenol and once with chloroform–isoamyl alcohol (24:1). Product precipitated at −20 °C for 48 h by addition of 2 volumes 100% ethanol. After centrifugation at 10,000*g* for 60 min, pellet resuspended in a minimal volume 0.1 M Tris-HCl, *p*H 8.0, and stored at −20 °C. After isopycnic centrifugation in neutral CsCl gradients, > 95% ^{3}H-radioactivity found associated with RNA.

*Hydrolysis by 7S Ig measured after incubation of 10 μl Ig and 5 μl ^{3}H-poly(A) (~ 1,500 c.p.m. per 5 μl) or poly(A)·^{3}H-poly(dT) (~ 1,000 c.p.m. per 5 μl) for 2 h at 4 °C and for 40 min at 37 °C. Mixture precipitated by addition of excess ice-cold 10% TCA. Acid-insoluble material collected on a 0.45-μm membrane filter (Millipore) and dried thoroughly. Unhydrolysed radioactivity measured in toluene-based scintillation fluid.

†10 μl Ig in 0.1 M Tris-HCl, *p*H 8.0, incubated with 5 μl ^{3}H-poly(A) or poly(A)·^{3}H-poly(dT) for 2 h at 0 °C and for 40 min at 37 °C. Ig precipitated by addition of 5 ml half-saturated ammonium sulphate solution. Precipitate centrifuged, washed with additional 5 ml half-saturated ammonium sulphate solution and centrifuged Amount of ^{3}H-label in precipitate determined and expressed as percentage total acid-precipitable input c.p.m.

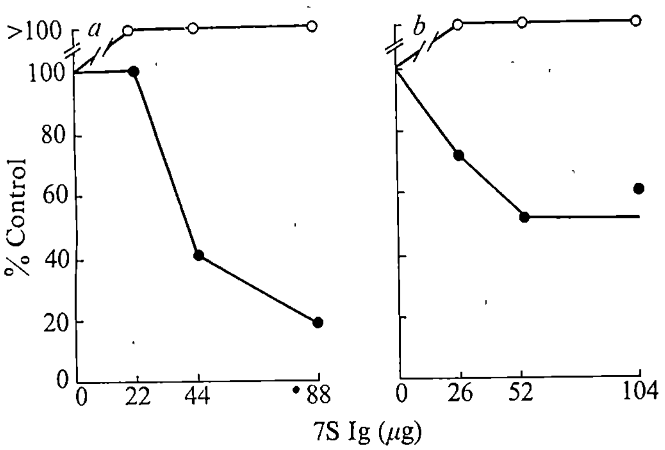

Fig. 3 Inhibition of purified reverse transcriptases by 7S Ig from patients M.R. (*a*) and R.M. (*b*). AMV (○) and SiSV (●) reverse transcriptases purified as described previously[15,16]. AMV enzyme, purchased from Schwarz-Mann; SiSV enzyme, gift of Drs R. E Gallagher and R. C. Gallo. Both enzymes stored at −20 °C in 0.26 M KCl, 30 mM Tris-HCl, *p*H 7.9, 0.6 mM dithiothreitol, 0.06% Sterox SL, and 50% glycerol. 40 μl 7S Ig in 0.1 M Tris-HCl, *p*H 8 0, incubated with 10 μl enzyme for 2 h at 4 °C with constant mild agitation. Reverse transcriptase reagents (150 μl) were added (see Table 1) and mixture incubated for 40 min at 37 °C. Each point determined from average of triplicate samples and expressed relative to average of six identical control samples containing enzyme in absence of 7S Ig.

the selective inhibition was the result of differential accessibility of the reverse transcriptases of primate viruses or of a nonspecific inhibition. The results indicate that some adult patients with leukaemia mount a humoral immune response against two antigenically distinct reverse transcriptase or against an enzyme which shares determinants with both SiSV and BEV enzymes. Although our observations are consistent with findings of SiSV- and BEV-related polypeptides in certain human tissues[20,22,26-28], our patient population is obviously too small to conclude that the humoral response to reverse transcriptase is confined strictly to patients with leukaemia.

We thank Drs D. Gillespie, R. Gallagher, R. Gallo, S. Panem, R. Rothberg and C. Rieger for discussions. Supported by the Renee Shaffer Gettleman Memorial Foundation Auxiliary and the US Public Health Service (National Cancer Institute). E.V.P. is a predoctoral student supported by the Medical Scientist Training Program (NIGMS).

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Received November 24; accepted December 18, 1975.

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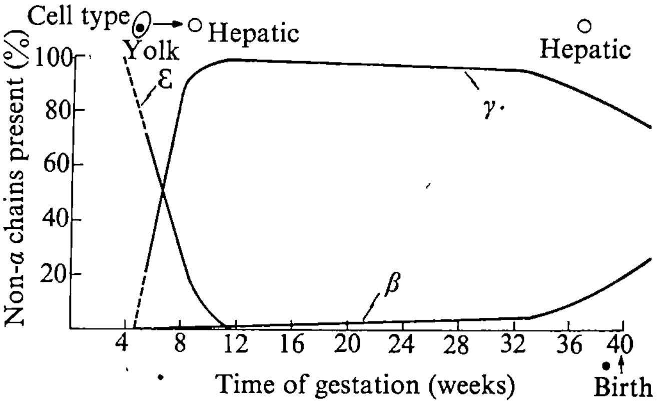
Increasing haemoglobin β-chain synthesis in foetal development is associated with a declining γ- to α-mRNA ratio

DURING human foetal development, the predominant haemoglobin in erythrocytes switches twice (Fig. 1). First, ε chains of embryonic haemoglobins in erythroblasts of yolk sac origin are replaced by γ chains of foetal haemoglobin and traces of β chains of adult haemoglobin. This switch occurs very early in foetal development, coincident with the appearance of a new type of peripheral erythrocyte of hepatic origin. Second, at about 32 weeks of gestation, predominantly γ-chain production gives way to increased β-chain synthesis within a constant cell type[1]. This double haemoglobin switch also occurs in other animals, including sheep[2]. Not only is very little known about the biological basis for the switch from γ- to β-chain production, but the basic changes in gene activity involved in the switching process are obscure. An understanding of the γ to β switch has clinical importance, for if one could block the switch and increase γ-chain production, sickle cell anaemia and β-thalassaemia could be treated effectively. We present here data concerning changes in globin gene activity, as assessed by globin mRNA levels, which are temporally related to the turning up of β-chain synthesis. These observations were originally made in erythroid cells of aborted human foetuses and were extended to cells from foetal sheep.

Globin mRNA of human sheep reticulocytes was isolated by oligo(dT)-cellulose chromatography of polyribosomal RNA[3,4]. Gould and Hamlyn demonstrated that the mRNA region of rabbit reticulocyte RNA contained two bands after acrylamide gel electrophoresis in formamide[5], and subsequent work in many laboratories demonstrated that the faster migrating of these RNAs is α mRNA and the slower is β mRNA[6-10]. This electrophoretic procedure separates the

Fig. 1 Percentage of non-α chains in foetal development plotted against time of gestation. The erythroid cell type, yolk sac or hepatic, is shown above.



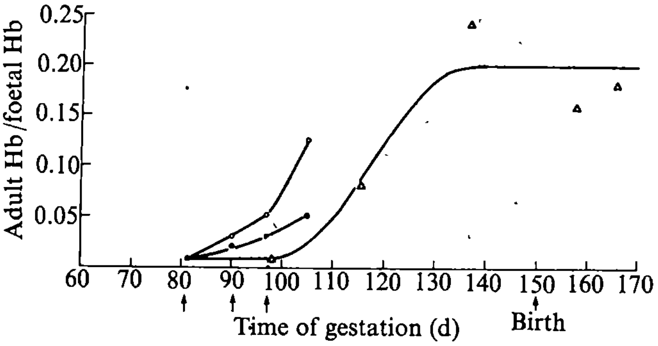


Fig. 2 Synthesis of adult haemoglobin (Hb) compared with that of foetal haemoglobin plotted against age of sheep from conception. The normal curve (△) is shown in addition to data obtained on two foetal sheep bled at times indicated by the arrows. ○, Sheep 5,283; ●, sheep 5,216. Erythroid cells of foetal sheep were washed and incubated with ^{35}S-methionine for 3 h (ref. 15). The haemoglobins were separated by Bio-Rex 70 chromatography as described for separation of human foetal and adult haemoglobins[15] except that the starting buffer was 0 019 M in sodium phosphate. Sheep haemoglobins A and B elute close to one another with starting buffer at 25 °C, and haemoglobin C elutes when the sodium phosphate concentration is increased to 0.058 M at 25 °C. Haemoglobin C synthesis was questionable except in sheep 5283 at 105 d when it accounted for nearly 5% of total haemoglobin synthesis.

α and β mRNAs in several species, including man[10,11], mouse[7], rabbit[6], sheep and goat (unpublished results of H.H.K. and P.G.S.). The same technique has shown that β mRNA is deficient in reticulocyte RNA preparations from human foetuses[11], and that in man, γ mRNA has an intermediate mobility between the β and α mRNAs[10–11].

In globin mRNA preparations from human adult reticulocytes, the ratio of β mRNA to α mRNA was determined by gel electrophoresis to be near 1 (ref. 11). Unfortunately, we have been unable to separate the γ and α mRNAs sufficiently to allow accurate estimation of the ratio of their concentrations in foetal cells

Therefore, we assayed foetal mRNA preparations in an ascites cell-free system[12] and compared the γ- to α-chain synthesis ratios with the β to α ratios obtained with mRNA from adult cells (Table 1). Although the cell-free system does not facilitate quantitation of the absolute ratios of chains synthesised, it makes possible the comparison of chain synthesis ratios directed by different mRNA preparations when mRNA is present in limiting concentrations. In this system the RNA from sickle cell patients and a 32-week premature infant gave β/α or γ/α ratios near unity. In contrast, assays of mRNA from mid-trimester foetuses resulted in γ/α-chain ratios much greater than 1. Thus, cell-free assays suggested that γ/α-mRNA ratios were greater than 1 at mid-trimester and near 1 at 32 weeks of gestation in man.

Table 1 Cell-free globin synthesis ratios

Human mRNA source	Chain synthesis ratio
	(β/α)
Adults (SS disease)	1.1
	1.0
	(γ/α)
32-week premature infant	1.4
Mid-trimester foetuses	4.0
	4.5

The source of globin mRNA for these assays was reticulocyte polyribosomal RNA. Assay conditions were those previously described[12] and mRNA concentrations were less than 1 µg per 60 µl, well below the concentration of 4–6 µg mRNA per 60 µl, which is non-limiting in our laboratory. Product was analysed by chain separation on Cellogel electrophoresis[16]. In addition, a γ/α-synthetic ratio of 2.1 was obtained in a mid-trimester foetal sample analysed by peptide mapping which is twice the β/α ratio observed in assays of adult mRNA preparations analysed in this manner.

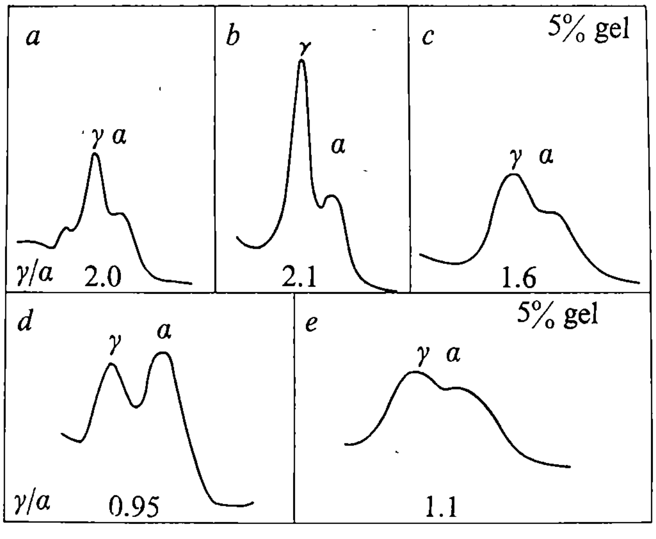
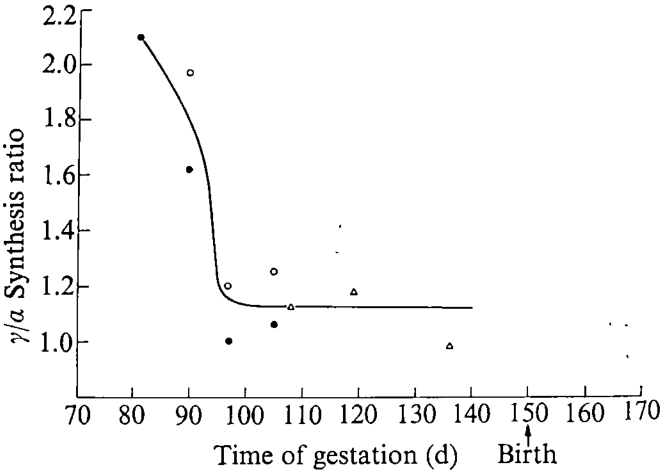


Fig. 3 Gel scans of globin mRNAs of foetal sheep at: *a*, 74 d; *b*, 81 d; *c*, 95 d; *d*, 98 d; *e*, 115 d of gestation. Reticulocytes were washed and lysed, and polyribosomes were obtained by ultracentrifugation[3]. The RNA solution was extracted, chromatographed on oligo(dT)-cellulose[4], and the mRNA fraction was subjected to electrophoresis in formamide on 7% polyacrylamide gels[6]. Scans of the gels after treatment with Stains-All were performed at 600 nm. Results shown in (*c*) and (*e*) were obtained with 5% polyacrylamide gels.

To provide further evidence for a declining γ/α-mRNA ratio in late foetal development, we used cells from foetal sheep. This animal has the following advantages. (1) Haemoglobin switching during development is very similar to that in man. At birth, adult haemoglobin accounts for 5–10% of total haemoglobin[2]. (2) Foetal sheep can be obtained at known gestational ages. (3) The foetus can be removed surgically from the uterus, manipulated and returned to the uterus without significant mortality.

Fig. 4 The γ/α synthesis ratio of foetal sheep reticulocytes plotted against time of gestation. Reticulocytes were incubated with ^{3}H-leucine and the radioactive chains were separated by Cellogel electrophoresis in urea and mercaptoethanol[16]. The relative amounts of γ and α chains recovered after electrophoresis and staining were determined by the absorbancy of dissolved Cellogel strips and the data were used to correct the d.p.m. associated with the γ and α chains for the yield of each chain. Results obtained with sheep foetuses that were not chronically bled are indicated by triangles. Bled foetuses were: ●, 5,283; ○, 5,216.



Blood samples were obtained from foetal sheep at various times during gestation, and haemoglobin synthesis, together with globin mRNA quantities, was studied in the cells. The ratio of adult haemoglobin synthesis to foetal haemoglobin synthesis was determined at various times of gestation (Fig. 2). The synthesis of adult haemoglobin is about 1% that of foetal haemoglobin until about 100 d of foetal life, which is two-thirds of the way through gestation. It then rises to 20% that of foetal haemoglobin by 130–140 d of gestation.

When globin mRNA of foetal sheep is subjected to formamide gel electrophoresis, unlike the situation in man, the non-α mRNA (the presumed γ mRNA) separates quite well from α mRNA (Fig. 3). When these two RNAs were eluted separately from gels in which the RNA separation was suboptimal, the slower migrating band directed the synthesis of 1.6 times as many γ chains as α chains in the cell-free system, while the faster migrating band directed the synthesis of a twofold excess of α chains. Sheep β mRNA has an electrophoretic mobility similar to that of γ mRNA by this technique But β-chain synthesis accounts for less than 10% of non-α chain synthesis up to 115 d of gestation, indicating that γ mRNA should be free of significant β-mRNA contamination until late in gestation. The ratio of γ mRNA to α mRNA was about 2 in reticulocytes at 70–80 d and then declined to near 1 at 98–100 d of gestation. Although it remained in this general range, a sample obtained at 145 d had a (γ mRNA+β mRNA) to α mRNA ratio of 1.3.

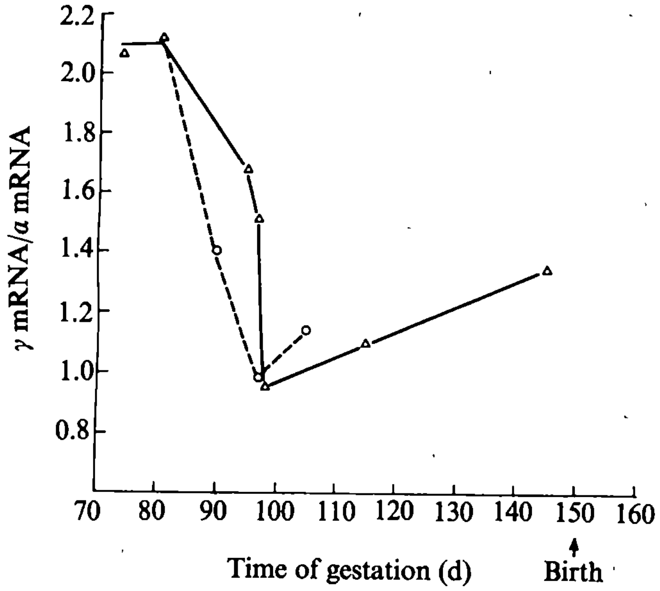


Fig. 5 The γ/α-mRNA ratio plotted against the time of gestation. Results from normal (△) and chronically bled foetuses (○) are shown. Blood from the two foetuses undergoing bleeding was pooled at each point to ensure sufficient mRNA for analysis.

To determine whether the decline in γ/α-mRNA ratio is important for the subsequent turning up of β-chain synthesis, we attempted to alter the timing of increased β-chain synthesis by chronic bleeding of foetal sheep. Two foetuses were bled of approximately one-fifth of their blood volume (40 ml) at 81, 90 and 97 d, and then bled and killed at 105 d of gestation. Haemoglobin and globin chain synthesis by reticulocytes, and globin mRNA levels were studied at these times. In one bled foetal sheep, the synthesis of adult haemoglobin increased prematurely, reaching 12.5% that of foetal haemoglobin at 105 d of gestation (Fig. 2). Adult haemoglobin synthesis in untreated foetuses at that gestational age is about 3% of foetal haemoglobin synthesis. In the second sheep, adult haemoglobin synthesis was also increased, but the moderate rise observed may not be significantly different from normal.

We then asked whether the premature increase in β-chain synthesis was associated with premature changes in γ/α mRNA and γ/α-chain synthesis ratios. In Fig. 4 the reticulocyte γ/α-chain synthesis ratio is plotted against the time of gestation in the bled foetuses. This ratio was about 2 at 80 d of gestation, suggesting that in the sheep translational controls do not operate to balance the synthesis of α and γ chains in the early foetus. In contrast, synthesis of α and γ chains during early foetal life in man is balanced[13]. Moreover, the γ/α-chain synthesis ratio dropped to 1 at about 90–100 d of gestation Just as the ratio of γ/α-chain synthesis declined at 90 d of gestation, so the γ/α-mRNA ratio also declined precipitously between 90 and 100 d of foetal age in both normal foetuses and bled foetal sheep (Fig. 5).

These studies of the turning up of β-chain synthesis suggest the following conclusions. First, β-chain switching in the sheep may be hastened by continued bleeding of the foetus. Whether this premature turning up of β-chain synthesis is a response to stress, hypoxia or other factors is not clear. Erythroprotein was not detectable in the blood of the bled foetuses until the day of death when it was 0.2 U ml^{-1} in one sheep (denoted 5,283, data of J. L. Spivak). Second, the γ/α-mRNA ratio is greater than 1 early in foetal development and decreases to unity around the time of increased β-chain production.

These data and the fact that the number of γ genes in man is at least twice that of β genes[1,14], suggest the following hypotheses. (1) Active γ-chain genes lead to a γ/α-mRNA ratio of 2 in the foetus, in contrast to the β/α-mRNA ratio of 1 in the adult. (2) This ratio is maintained until about 30 weeks of gestation in man and 90 d of gestation in sheep when transcription of γ-chain genes declines, leading finally to a partial deficiency of γ chains within precursor erythroid cells, which is followed by an increased production of β chains in these cells. The data presented here indicate that a relative decline in γ mRNA occurs in reticulocytes at about the time of increased β-chain synthesis, but they do not demonstrate that this decline is a necessary precedent to the turning up of β-chain production. In these experiments a premature increase of β-chain synthesis was produced in two bled foetal sheep, but a premature decline in the γ/α-mRNA ratio and the parallel γ/α-chain synthesis ratio, which the data suggest, requires further investigation.

This work was supported in part by a contract and grants from the National Institutes of Health, and by an Independent Order of Odd Fellows Research Professorship (A.M.S.). We thank Shirley L. Johnson for technical assistance.

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Received November 3, accepted December 12, 1975

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Half life of yeast messenger RNA

WE have determined the half life of *Saccharomyces cerevisiae* messenger RNA (mRNA) and its associated poly(A) sequences, from the kinetics of their labelling with ^{14}C-adenine. Corrections have been made for changes in the ATP pool specific activity during the labelling period. This method of determining the mRNA half life is preferred to observing the decay of mRNA in the presence of inhibitors because changes in the cell metabolism caused by an inhibitor may also change the mRNA half life[1]. One of our purposes in determining these half lives is for their use in the characterisation of temperature-sensitive mutants which are defective in macromolecular synthesis[2]. For this reason we have determined the half lives of mRNA and poly(A) in two different conditions, at 23 and 36 °C.

The incorporation of ^{14}C-adenine into mRNA has been followed in total mRNA and in the poly(A) portion of mRNA. The total mRNA was obtained from polysomes isolated on sucrose gradients. To separate rRNA and mRNA, the polysome fractions were collected and rerun on 10–30% sucrose gradients in low magnesium buffer, in which the polysomes dissociate into 40S and 60S subunits and mRNA. Figure 1 shows a typical subunit gradient, in this case from polysomes labelled for 30 min at 36 °C with ^{14}C-adenine. The rRNA in the 60S and 40S subunits is clearly resolved. The mRNA forms a broad peak that, for the most part, sediments more slowly than the 40S subunit. The division between counts in mRNA and subunits is estimated by assuming that the specific activity of the subunit RNA is constant across the peak. (The tRNA accounts for < 3% of the radioactivity in the ribosomal subunits.) The radioactivity in mRNA is determined from these gradients and normalised to the absorption in total ribosomal RNA on the gradients, to correct for variations in the extent of cell lysis in the sample. These data are plotted in Fig. 2. The curve drawn through the points is a computer fit of equation (3), as is discussed below.

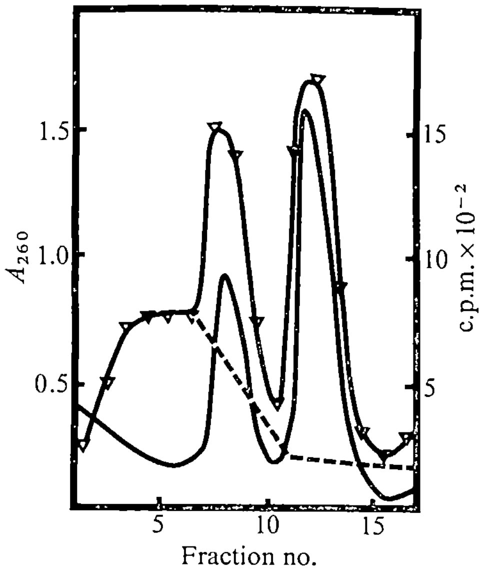


Fig. 1 Dissociation of polysomes into ribosomal subunits and mRNA. Cells were converted to spheroplasts[12], which were labelled with ^{14}C-adenine for the indicated time, lysed, and layered on sucrose gradients containing 0.03 Mg^{2+} (ref. 12) The polysome region (as determined by an ultraviolet scan) from these gradients was pooled and centrifuged at 160,000*g* for 3 h and the pellet resuspended in S buffer (0.01 M Tris, 0.1 M NaCl, 10^{-5} M $MgCl_2$, *p*H 7.4) to dissociate the polyribosomes This was layered on a 15–30% sucrose gradient in S buffer and centrifuged at 90,000*g* for 17 h. The gradient shown is representative, and is from a culture labelled for 30 min at 36 °C. ▽, c p.m. in ^{14}C-adenine. The theoretical curves for mRNA (- - -) and rRNA (——) are also shown

Table 1 Ratio of ^{3}H/^{14}C in intercellular pools*

	ATP			
	23 °C		36 °C	
Time labelled with ^{3}H-adenine (min)	Average value	Mean deviation	Average value	Mean deviation
5	0.41	0.01	0.38	0.04
15	0.83	0.12	0.88	0.01
30	1.00		1.00	

*Spheroplasts were prepared as in Fig. 2 and allowed to grow for 3 h in media labelled with 0.5 µCi ml^{-1} ^{14}C-adenine (specific activity 45 mCi mmol^{-1}). 30 µCi ml^{-1} ^{3}H-adenine (10 Ci mmol^{-1}) was added, and 1-ml samples were collected in equal volumes of 10% TCA. The TCA samples were filtered, and the filtrate was charcoal absorbed and washed as described in ref. 9, except that the mixture used to elute the nucleotides from the charcoal was ethanol–28% NH_4OH–H_2O (65:2:32 v/v). The samples were dissolved in water with carrier ATP, GTP, UTP, and CTP, and were spotted on thin-layer PEI cellulose plates (Brinkman, CEL 300 PEI, 20×20 cm). The solvent system used to separate the trinucleotides was developed by Neuhard *et al.*[10] and modified by Mowbray[11]. Plates were developed 3 cm in 2 M LiCl–2 M acetic acid (1:1 v/v) and then to 15 cm in 2.5 M LiCl–2 M acetic acid (1:1 v/v) in the first dimension. They were dried, washed in methanol, and developed in the second dimension first to 5 cm in 3 M ammonium acetate, 3.6% boric acid *p*H 7.0 and then to 15 cm in 4 M ammonium acetate, 5% boric acid *p*H 7.0. The plates were dried and the ATP and GTP spots identified by autoradiography and the ultraviolet absorption of the markers. These spots were cut out and counted to determine the ^{3}H/^{14}C ratio.

The half life of poly(A) in polysomes was determined by following the incorporation of ^{14}C-adenine into nuclease-resistant material, isolated from polysomes, which binds to poly(dT)– cellulose columns[4]. These data were fitted in the same way as total mRNA (Fig. 3).

Table 2 Decay constants of yeast RNA*

	mRNA			Poly(A)		
	µ	*k*	Half life	µ	*k*	Half life
23°C	0.0416	0.115	16.6	0.0601	0.115	11.5
36°C	0.0421	0.128	16.4	0.0547	0.128	12 6

*Values have been obtained from the computer fit of the data shown in Figs 2 and 3 and are corrected for the labelling of the ATP precursor. The values for the half lives of mRNA and the associated poly(A) sequences, uncorrected for the labelling kinetics of the ATP pool, were ~ 9 min longer in each case.

The radioactivity in mRNA as a function of time depends on the specific activity of the ATP precursor pools as well as the rate of synthesis and destruction of mRNA. If the change in specific activity of the ATP pools was neglected, the amount of mRNA after short times would be underestimated relative to the amount made after longer periods of labelling. We measured the specific activity of the ATP pool in cultures labelled identically to those used for the determinations of half life using two-dimensional thin-layer chromatography to isolate cellular nucleoside triphosphates (Table 1). Since the conversion of adenine to guanine is restricted by the presence of non-radioactive guanine in the medium, the labelling of GTP was ~ 10% of that of ATP. This yielded, given the relative sizes of the ATP and GTP pools[3], a specific activity of the GTP of < 30% of that of the ATP at the longest labelling times. The evolution of this rise in specific activity, in so far as could be measured (data not shown), followed that of the ATP. For these reasons a correction for the kinetics of labelling of the GTP was not necessary.

We have fitted our data for the labelling of mRNA (Figs 2 and 3) to an equation which takes into account changes in ATP specific activity. We assume that the specific activity of the mRNA is a function of the specific activity of the ATP pool and of the rate of synthesis and degradation of mRNA

$$\frac{dM}{dt} = \mu(P-M) \qquad (1)$$

where M is the specific activity of the messenger pool, μ is the rate of synthesis of mRNA per unit of mRNA in the cell, and P is the specific activity of the ATP pool The assumptions involved in this equation are that the amount of mRNA per unit cell mass is constant, and that the decay of mRNA is random. To obtain values for P we have fitted the data for the specific activity of the ATP pools to a curve of the form

$$P = 1-\exp(-kt) \qquad (2)$$

where k is the time constant of labelling of the ATP pool and the final specific activity of the ATP pool is defined as 1.0. Using equations (1) and (2) and solving for M

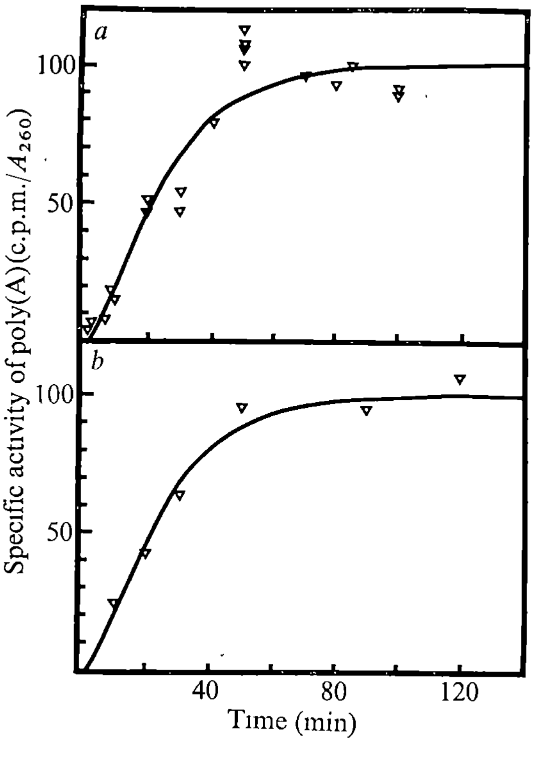
$$M = 1+\left[\frac{k\exp(-\mu t)-\mu\exp(-kt)}{\mu-k}\right] \qquad (3)$$

When the specific activity of the pool is constant, the solution to equation (1) is

$$M = 1-\exp(-\mu t) \qquad (4)$$

Table 2 gives the values of k and μ for each of the curves in Figs 2 and 3 as well as the half lives of the RNA. The half life of total mRNA is ~ 16 min at both 23 and 36 °C whereas that of poly(A) is ~ 12 min at both 23 and 36 °C. This difference of ~ 4 min in the half lives of mRNA and poly(A) could arise from: first, differences in the labelling of internal precursor pools from which poly(A) and mRNA are made; second, clipping of poly(A) during the lifetime of the messenger so that an older message would have shorter poly(A) sequences or third, the presence of a longer lived fraction of mRNA that contains no poly(A).

Fig. 2 The kinetics of ^{14}C-adenine incorporation into mRNA. The curve is a least squares computer fit of the data points to equation (3) with k determined from the data in Table 1 The specific activity of mRNA is normalised to 100 for maximum labelling a, mRNA labelled at 23 °C; b, mRNA labelled at 36 °C.



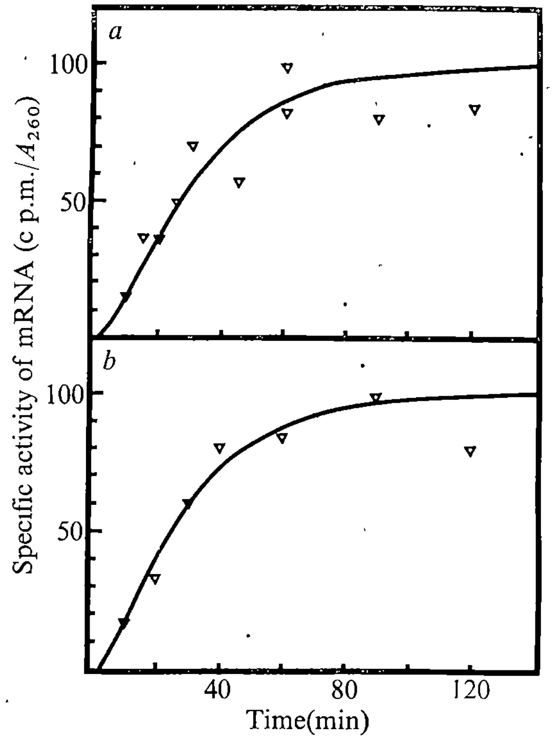


Fig. 3 The kinetics of ^{14}C-adenine incorporation into messenger-associated poly(A). The curve is a least squares computer fit of the data points to equation (3), with k determined from the data in Table 1. a, Poly(A) labelled at 23 °C; b, poly(A) labelled at 36 °C.

There have been several previous determinations of yeast mRNA half life. Hartwell *et al.* have measured the kinetics of incorporation of ^{14}C-adenine into polysomes in the presence of cyclohexamide and the decay of polysomes in the mutant *rna 1* which is defective in cytoplasmic RNA production[5]. Also, Tønneson and Friesen have followed the decay of protein synthesis in the presence of daunomycin or ethidium bromide[6]. The value obtained for the half life of mRNA in these experiments was ~ 22 min.

The value we obtained for the half life of the mRNA in yeast is 10–15% of the cell doubling time. This is intermediate between the values obtained for *E. coli* of ~ 3–6% of the doubling time, and values obtained for HeLa cells and mouse C cells of 70% or more of the cell doubling time[7,8].

We thank Joy Barnitz, Robert Petersen and Pamela Sutherland for help. This investigation was supported by Public Health Service grants from the National Institute of General Medical Sciences and a grant from the National Cancer Institute.

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Aminoacylation and nucleoside modification of *in vitro* synthesised transfer RNA

TRANSCRIPTION of transfer RNA (tRNA) genes *in vitro* by purified RNA polymerase has the advantage of producing completely unmodified tRNA precursors which provide a substrate for the study of the processes and modifications leading to the formation of mature tRNA molecules. We have shown[1] that the tRNA genes, *tRNA$_1^{Tyr}$* (*su$_3^+$* and *su$_3^-$*), *tRNA$_2^{Gly}$ su$^+$A36*, *tRNA$_3^{Thr}$* and *tRNA$_2^{Tyr}$* (carried by the respective φ80psu$_3^{+,-}$ and λh80T phage DNA[2,3]), were transcribed *in vitro* by *Escherichia coli* RNA polymerase into polycistronic tRNA precursors. These precursors were cleaved on incubation with a supernatant fraction from *E. coli*, yielding mature size tRNA molecules[1]. The transcription *in vitro* of an active *tRNA$_1^{Tyr}$ su$_3^+$* by crude S-30 *E. coli* extracts or a combination of partially purified fractions has been described[4–6]. The formation of the primary transcription products of the *tRNA^{Tyr}* gene was not, however, clearly demonstrated and it was concluded that an additional *E. coli* extract fraction would be required to enable purified RNA polymerase to function in tRNA synthesis[5,6]. We report here evidence indicating that the *in vitro* transcription of φ80psu$_3^{+,-}$ or λh80T DNA by purified *E. coli* RNA polymerase alone produces tRNA precursor molecules which are not only processed to form mature-size tRNA[1] but are also recognised by specific modifying enzymes. In addition the tRNA molecules (tRNA$_1^{Tyr}$, tRNA$_2^{Tyr}$, tRNA$_3^{Thr}$ and tRNA$_2^{Gly}$) synthesised and processed *in vitro*, possess amino acid acceptance activity.

For the study of the formation of modified nucleosides in *in vitro* synthesised tRNA, φ80psu$_3^{+,-}$ and λh80T DNA were transcribed by *E. coli* RNA polymerase in reaction mixtures containing α-^{32}P-CTP, α-^{32}P-UTP or α-^{32}P-ATP. After 30 min incubation at 37 °C RNA synthesis was terminated by addition of actinomycin D and DNase I. Synthesised RNA was processed by incubation for 90 min at 37 °C with a dialysed *E. coli* S-100 extract. RNA was extracted with phenol, precipitated using ethanol and fractionated by electrophoresis on a 5% polyacrylamide–7 M urea gel, as described previously[1]. tRNAs were located by autoradiography, extracted from the gel and digested by T$_1$ or T$_2$ RNase. Modified nucleosides, obtained as a result of the enzymatic activities present in S-100 extracts, were identified by two-dimensional thin-layer chromatography of the 3′ mononucleotides from T$_2$ RNase hydrolysates. Alternatively, oligonucleotides were isolated from two-dimensional fingerprints of T$_1$ RNase digestion fragments, hydrolysed by alkali or T$_2$ RNase, and the modified nucleotides identified by paper chromatography[7].

Table 1 Levels of nucleotide modifications in *in vitro* synthesised tRNA

Modified nucleotide	Molar yield Expected	Molar yield Formed*
	For tRNA$_1^{Tyr}$ or tRNA$_2^{Tyr}$	
ψp	2	2
Tp	1	1
2′-OmeGp	1	—
G*p	1	—
s^{4}Up	1†	0.74‡
mes^{2}i^{6}Ap	1	0.62–0.75
	For tRNA^{Thr} tRNA^{Gly} (1:1 mixture)	
ψp	2	2
Tp	2	2
me^{7}Gp	1	0.75
t^{6}Ap	2	1.28§
Dp	1	—
U*p	1	—

*Molar yields of modified nucleotides determined by paper chromatography of T$_2$ RNase hydrolysates of the respective T$_1$ oligonucleotides extracted from fingerprints.

†In tRNA$_1^{Tyr}$ synthesised *in vitro* in presence of α-^{32}P-UTP, only one of the two s^{4}Up residues (U$_9$) is expected to be labelled by nearest-neighbour α-^{32}P-UMP distribution.

‡s^{4}U was measured in tRNA$_1^{Tyr}$ only.

§Molar yield of t^{6}A was determined from two-dimensional thin-layer chromatography as described in Fig. 3, assuming two residues in the 1:1 mixture of tRNA^{Thr} tRNA^{Gly}.

To study the formation of pseudouridine (ψ), the *in vitro* synthesised tRNAs were labelled by incorporation of α-^{32}P-CMP. tRNA$_1^{Tyr}$ and tRNA$_2^{Tyr}$ contain two ψ residues: one in the T$_1$ fragment TψCG and a second near the anticodon in the oligonucleotides ACUCUAAAψCUG or UAAAψCUG (Fig. 1). tRNA^{Thr} and tRNA^{Gly} contain one ψ each in the TψCG fragments. Digestion of the α-^{32}P-CMP-labelled oligonucleotides by alkali generated ^{32}P-labelled ψMP which was then identified by paper chromatography (Fig. 2). Results show that incubation of the primary transcription products with dialysed *E. coli* S-100 extracts, a complete modification of U to ψ occurs in the TψCG fragments derived from tRNA$_1^{Tyr}$ (Fig. 2*a*, strip 2), tRNA$_2^{Tyr}$, tRNA^{Thr} and tRNA^{Gly} (Fig. 2*b*, strips 1 and 3). Formation of ψ in the anticodon region of tRNA^{Tyr} was also quantitative

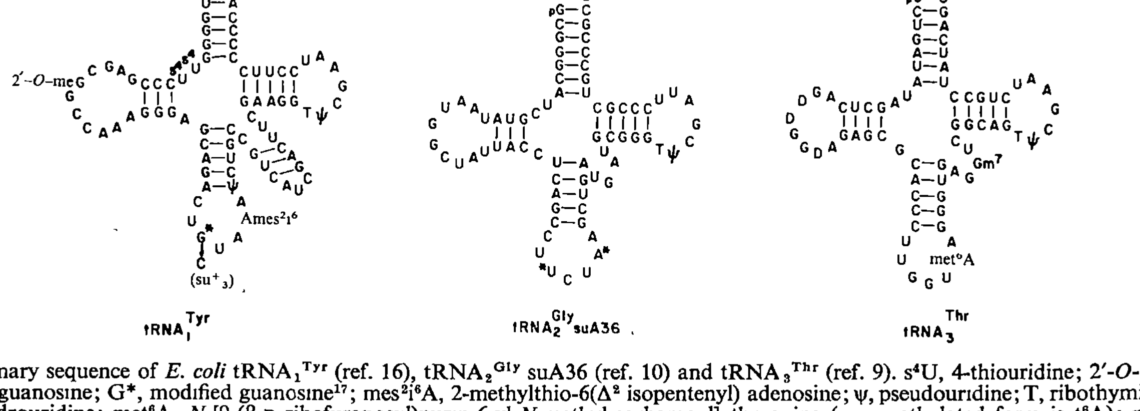

Fig. 1 Primary sequence of *E. coli* tRNA$_1^{Tyr}$ (ref. 16), tRNA$_2^{Gly}$ suA36 (ref. 10) and tRNA$_3^{Thr}$ (ref. 9). s^{4}U, 4-thiouridine; 2′-*O*-meG, 2′-*O*-methylguanosine; G*, modified guanosine[17]; mes^{2}i^{6}A, 2-methylthio-6(Δ^2 isopentenyl) adenosine; ψ, pseudouridine; T, ribothymidine; D, 5,6-dihydrouridine; met^{6}A, *N*-[9-(β-D-ribofuranosyl)purin-6-yl-*N*-methyl-carbamoyl] threonine (non-methylated form is t^{6}A); me^{7}G, 7-methylguanosine; U*, unidentified derivative of uridine; A*, derivative of t^{6}A.

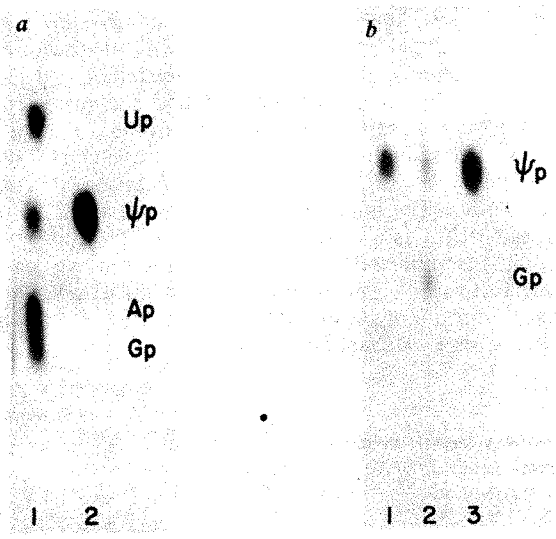

Fig. 2 Paper chromatography of hydrolysates of ψp-containing T$_1$ oligonucleotides from *in vitro* synthesised tRNAs. tRNA$_1^{Tyr}$, tRNA$_2^{Tyr}$, tRNA^{Gly} and tRNA^{Thr} were synthesised *in vitro* by transcribing 20 μg φ80psu$_3^{+,-}$ or λh80T DNA in 200 μl reaction mixtures containing 0.05 M Tris-HCl (*p*H 7.9), 0.01 M MgCl$_2$, 0.05 M KCl, 1 mM dithiothreitol, 0.4 mM each of ATP, GTP and UTP, 60 μM α-^{32}P-CTP (10.3 Ci mmol^{-1}; Amersham, England), 12 μg ρ termination factor and 160 U RNA polymerase (1,600 U mg^{-1}). Synthesis was initiated after 5 min preincubation at 38 °C by addition of nucleoside triphosphates. After 30 min incubation at 38 °C the reaction was stopped by addition of actinomycin D (20 μg) and DNase 1 (2 μg). The mixture was incubated with 400 μg dialysed S-100 extract for 90 min at 38 °C. Synthesised tRNAs were phenol extracted, purified by acrylamide gel electrophoresis, digested by T$_1$ RNase and the resulting oligonucleotides fractionated by two-dimensional electrophoresis as described previously[1]. ψp-containing oligonucleotides from fingerprints were extracted, alkali digested and analysed for nearest-neighbour distribution of α-^{32}P-CMP by paper chromatography in an isopropanol–HCl–H$_2$O (680 : 176 : 144 v/v) system[7]. *a*, Hydrolysates of (1) ACUCUAAAψCUG(C) and (2) TψCG derived from tRNA$_1^{Tyr}$ su$_3^+$; *b*, hydrolysates of (1) TψCG, (2) UAAAψCUG(C) derived from tRNA$_2^{Tyr}$, and (3) TψCG from tRNA^{Tyr} and tRNA^{Gly}. DNA-dependent RNA polymerase was prepared from *E. coli* MRE-600 cells[20] and further purified by low salt glycerol centrifugation[21]. Termination factor ρ (ref. 14) and S-100 extract[1] were prepared from *E. coli* MRE-600.

and the nearest-neighbour distribution of CMP was as expected —1 (ψp):1 (Up):1 (Ap): 1 (Gp) for ACUCUAAAψCUG(C) of tRNA$_1^{Tyr}$ su$_3^+$ and 1(ψp):1(Gp) for UAAAψCUG(C) of tRNA$_2^{Tyr}$ (Fig. 2*a*, strip 1 and Fig. 2*b*, strip 2).

From these results it may be concluded that the *in vitro* synthesised tRNA can serve as a substrate for the two enzymatic activities[8] responsible for the formation of ψ in the TψCG and in the anticodon regions of the tRNA. Formation of ψ is the only modification observed in the *in vitro* synthesised tRNAs as a result of incubation with dialysed S-100 extracts. To study the methylations leading to the formation of T and me^{7}G or the thiolation leading to the formation of s^{4}U, tRNA molecules were synthesised *in vitro* with α-^{32}P-UTP and incubated with dialysed S-100 extract in the presence of S-adenosyl-L-methionine or L-cysteine, respectively. Figure 3*a* shows the two-dimensional thin-layer chromatography of a T$_2$ RNase digest of tRNA^{Thr}, tRNA^{Gly} (1:1 tRNA mixture migrating as a single band on acrylamide gel[1]) in which the methylated nucleotides Tp and me^{7}Gp can be identified. Tp was also determined in the TψCG fragments isolated from two-dimensional fingerprints of T$_1$ RNase digests. Yield of Tp was found to increase with S-adenosyl-L-methionine concentration, reaching 100% at 1.15×10^{-4} M. Formation of me^{7}G was inferred also from the presence of the olignucleotide me^{7}GUCG in a two-dimensional fingerprint of the T$_1$ digest of α-^{32}P-UMP-labelled tRNA^{Thr} (data not shown). me^{7}G was identified by paper chromatography of a T$_2$ digest of the oligonucloetide, and an efficiency of 75% measured for me^{7}G synthesis.

Figure 3*b* shows the modification of U$_9$ of tRNA$_1^{Tyr}$ to s^{4}U (yield 74%). The synthesis of the hypermodified bases mes^{2}i^{6}A (in tRNA$_1^{Tyr}$ and tRNA$_2^{Tyr}$) and t^{6}A (in tRNA^{Thr}, tRNA^{Gly}) is illustrated in Fig. 3*c* and *d*. tRNAs were synthesised with α-^{32}P-ATP and incubated with S-100. extracts in the presence of specific cofactors as described in Fig. 3*c* and *d*. A yield of 62–75% was observed for mes^{2}i^{6}A and 64% for t^{6}A modifications of the specific A residues. Note, however, that A$_{37}$ in tRNA^{Thr} was modified only to t^{6}A and was not further methylated to met^{6}A as expected from the studies of Clarke and Carbon[9]. In addition it was observed that the molar yield of t^{6}A in the *in vitro* synthesised tRNA^{Thr} tRNA^{Gly} mixture exceeded the expected value for a single t^{6}A residue present in tRNA^{Thr}, thus supporting data of Roberts and Carbon[10] showing the presence of a t^{6}A derivative at A$_{37}$ in tRNA^{Gly} *su$^+$A36*. The molar yields of the nucleotide modifications obtained in *in vitro* synthesised tRNA molecules are summarised in Table 1.

To test the amino acid accepting ability of the *in vitro* synthesised tRNA molecules, φ80psu$_3^{+,-}$ and λh80T DNAs were transcribed by purified RNA polymerase in the presence or absence of termination factor ρ and the synthesised tRNA precursors were processed by a tRNA-free dialysed *E. coli* extract. The resulting tRNAs were extracted with phenol, precipitated using ethanol and assayed for acceptance of ^{3}H-tyrosine, ^{3}H-threonine and ^{3}H-glycine. Figure 4 shows the linearity of aminoacyl-tRNA formation as a function of *in vitro* synthesised tRNA. Note the enhancing effect of ρ, when present

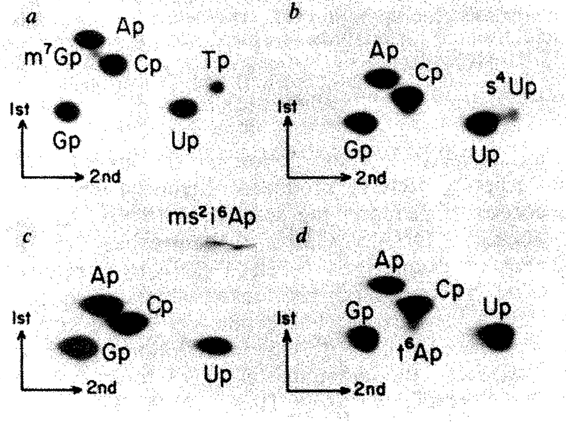

Fig. 3 Autoradiography of two-dimensional thin-layer chromatography of T$_2$ digests of *in vitro* synthesised and modified tRNAs. *a*, α-^{32}P-UMP-labelled tRNA^{Thr} tRNA^{Gly} mixture (1:1) was synthesised *in vitro* by transcription of λh80T DNA, followed by 90-min incubation with 2.2 mg ml^{-1} dialysed *E. coli* S-100 extract, 0.12 mM S-adenosyl-L-methionine and 0.6 mM EDTA. *b*, α-^{32}P-UMP-labelled tRNA$_1^{Tyr}$ was synthesised by transcription of φ80psu$_3^{+,-}$ DNA followed by 90-min incubation with 2.2 mg ml^{-1}dialysed *E. coli* S-100 extract, 1 mM L-cysteine, 5 mM ATP, 0.05 mM pyridoxal phosphate and 10 mM β-mercaptoethanol. α-^{32}P-ATP-labelled tRNA$_2^{Tyr}$ (c) and α-^{32}P-ATP-labelled tRNA^{Thr}/tRNA^{Gly} (*d*) were synthesised by transcription of λh80T DNA followed by 90 min incubation with 4.4 mg ml^{-1} non-dialysed *E. coli* S-100 extract, (0.2 mM) Δ^3-isopentenyl pyrophosphate (Fox chemicals), 4 mM ATP, 1 mM L-cysteine, 0.12 mM S-adenosyl-L-methionine, 10 mM β-mercaptoethanol, 450 μg ml^{-1} isopentenylpyrophosphate isomerase[18], 0.4 mM L-threonine and 25 μg ml^{-1} folinic acid. tRNAs were extracted with phenol, precipitated using ethanol, separated by acrylamide gel electrophoresis and extracted as described previously[1]. T$_2$ RNase hydrolysis was as described by Barrell[7] and the two-dimensional chromatography followed the method of Nishimura[19]. Molar yields of modified nucleotides were determined by summing radioactivity found in all the spots from the respective plate. This number was normalised to the expected number of moles of nucleotides in a nearest-neighbour position to the incorporated α-^{32}P-UMP, -AMP or -CMP as determined from the primary sequence of the respective tRNA (Fig. 1). The fraction of radioactivity in the modified nucleotide spot related to the calculated radioactivity for a single nucleotide gives the molar yield of modification.

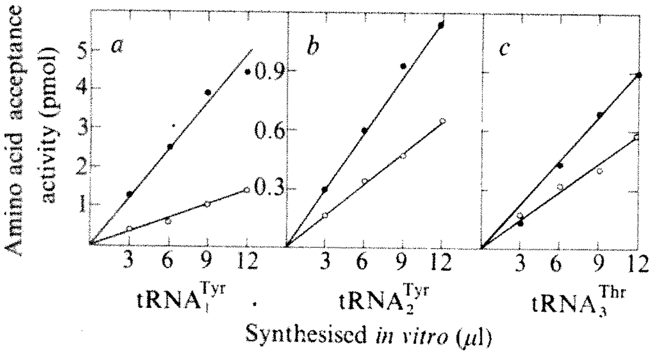

Fig 4. Amino acid acceptance of *in vitro* synthesised tRNAs. $tRNA_1^{Tyr}$ (*a*), $tRNA_2^{Tyr}$ (*b*) and $tRNA^{Thr}$ (*c*) were synthesised *in vitro* in the presence (●) or absence (○) of 12 μg ρ factor in 200 μl reaction mixtures containing 0.05 M Tris-HCl (*p*H 7.9), 0.01 M $MgCl_2$, 0,05 M KCl, 1 mM dithiothreitol, 0.4 mM each of ATP, CTP, UTP and GTP, 20 μg $\varphi 80psu_3^{+,-}$ DNA or 30 μg λh80T DNA, and 160 U RNA polymerase (1,600 U mg^{-1}). Synthesis was initiated after 5 min preincubation at 38 °C by addition of nucleoside triphosphates. After 30 min incubation at 38 °C the reaction was stopped by addition of 20 μg actinomycin D and 2 μg DNase I. tRNA-free S-100 extract (320 μg) was added and the incubation continued for 90 min. Synthesised tRNA was extracted with phenol, precipitated using ethanol and dissolved in 45 μl water. Aliquots of 3, 6, 9 and 12 μl were assayed for amino acid acceptance in 50 μl reaction mixtures containing 0.04 M Tris-HCl (*p*H 7.5), 0.01 M $MgCl_2$, 2 mM ATP, 3 mM glutathion, 1.5 μM L-3.5-^3H-tyrosine (41 Ci $mmol^{-1}$, Amersham UK) or 11 μM L-^3H-threonine 1.8 Ci $mmol^{-1}$ (New England Nuclear), 19 unlabelled amino acids (except tyrosine or except threonine respectively) and purified tyrosyl-tRNA synthetase[22] or 16 μg tRNA-free S-100 extract containing threonyl-tRNA synthetase. Incorporation of ^3H-labelled amino acids into acid-insoluble material was measured after 10 min incubation at 37 °C. tRNA-free S-100 extract was prepared by passing an *E. coli* S-100 extract adjusted to 0.4 M KCl through a DEAE–cellulose column.

during tRNA gene transcription on the amounts of amino acid-accepting tRNAs produced. Figure 4 shows that the presence of ρ increases the amount of $tRNA_1^{Tyr}$ transcribed on $\varphi 80psu_3^{+,-}$ DNA by a factor of 3–4 and that of the $tRNA_2^{Tyr}$ and $tRNA^{Thr}$ transcribed on λh80T DNA by a factor of 1.5–2. These results confirm our previous observations concerning the effect of ρ factor on tRNA synthesis[11,12]. We have shown[13] that the presence of ρ during transcription of the $tRNA_1^{Tyr}$ gene increases the amounts of tRNA precursor produced without affecting its size. Thus, although it is generally assumed that ρ factor affects only the termination of an RNA molecule[14] our results do not indicate a termination effect for ρ in tRNA gene transcription. Note that transcription of tRNA genes by purified RNA polymerase also takes place in the absence of ρ and does not require any additional factor as suggested by Bikoff and Gefter[5,6].

The efficiency of production of amino acid chargeable tRNA molecules in the *in vitro* system is remarkable. From the data reported in the legend to Fig. 4 it can be calculated that the transcription of approximately 1.4 pmol $tRNA_1^{Tyr}$ gene DNA (20 μg $\varphi 80psu_3^{+,-}$ DNA of molecular weight 30×10^6, containing 2 $tRNA_1^{Tyr}$ genes) resulted in the formation of 5.6 and 19.5 pmol $tRNA_1^{Tyr}$ when synthesised in the absence or presence of ρ respectively. Transcription *in vitro* of λh80T DNA was found to produce $tRNA_2^{Tyr}$ and $tRNA^{Thr}$ in equivalent amounts as determined by their amino acid acceptor activities (Fig. 4*b* and *c*). The glycine-accepting capacity of synthesised $tRNA^{Gly}$ was only 50% of that measured for $tRNA_2^{Tyr}$ or $tRNA^{Thr}$ (data not shown). This is probably due to the lower affinity of glycyl-tRNA synthetase for the $tRNA_2^{Gly}su^+A36$ species[15].

From the present *in vitro* studies we conclude that purified RNA polymerase alone can efficiently transcribe tRNA genes to produce primary tRNA percursors. The high fidelity of the transcription is illustrated by the fact that the synthesised RNA molecules are recognised as substrates by processing nucleases, by nucleoside modifying enzymes and by the aminoacyl-tRNA synthetases. Note that tRNAs synthesised *in vitro*, when processed by dialysed S-100 extracts, are (with the exception of ψ formation) completely unmodified. The unmodified tRNA precursors can be correctly processed and the resulting unmodified tRNAs are capable of charging with the corresponding amino acid. These findings argue against a possible role of the modified bases in tRNA precursor processing or tRNA aminoacylation. The present study shows that the *in vitro* synthesised tRNA is a suitable substrate for the study of the enzymes involved in nucleoside modifications and may serve to elucidate further the role of the modified nucleosides in the biological functions of mature tRNA.

We thank Mr Y. Tichauer for assistance.

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Received November 18; accepted December 31, 1975.

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Evolutionary origin of 5.8S ribosomal RNA

IN prokaryotes, the genes for 5S, 16S and 23S ribosomal RNA are linked in a polycistronic transcriptional unit[1,2]. In eukaryotes, on the other hand, the analogous unit contains the genes for 5.8S, 18S and 28S rRNAs, whereas the 5S RNA genes are found elsewhere in the genome. The analogous positions occupied by prokaryotic 5S RNA genes and eukaryotic 5.8S RNA in their respective genomes prompted Doolittle and Pace to speculate that these two RNA classes, both of which are components of the large ribosomal subunit, might have a common evolutionary origin[3]. If these RNAs are in fact homologous, one would expect their sequences to bear some relationship to each other. We report here our evaluation of possible structural relationships between known sequences of 5.8S and 5S rRNA, and conclude that they have no common evolutionary origin.

The comparison of RNA sequences is extremely difficult when insertions and deletions have to be considered. Recently, however, we have developed a test of similarity which can be applied to RNA sequences and which involves the establishment of the best alignment (most identities) of two sequences allowing a fixed number of insertions/deletions[4]. The number of positions in the alignment which are occupied by identical bases in the two sequences is counted. The process is repeated, increasing the allowed number of insertions/deletions by one. The number of identities are then compared with like values computed

Table 1 Comparison of 5S and 5.8S RNA sequences

Experiment	RNA	Insertions or deletions	Identities	Identities in random sequences
1	5.8S yeast 5.8S hepatoma	2	112	61
2	5.8S yeast 5S *E. coli*	8	67	66
3	5.8S yeast 5S KB cells	8	66	65
4	5.8S yeast 5S protoprokaryote	8	62	60
5	5.8S yeast 5S protoeukaryote	8	58	61
6	5.8S hepatoma 5S *E. coli*	8	67	69
7	5.8S hepatoma 5S KB cells	8	63	66
8	5.8S hepatoma 5S protoprokaryote	8	61	61
9	5.8S hepatoma 5S protoeukaryote	8	59	64

*The number of identities between random sequences is an average of five different experiments. Random sequences have the same composition and length as the two real sequences being compared in each experiment. We have chosen to give the number of identities found permitting eight insertions or deletions, since these are representative of values obtained permitting either more or less.

from random sequences. Related sequences show statistically significant increases in the number of identities found for each increase in the number of insertions/deletions permitted, up to a certain limit.

Previously, we have shown that 5S RNAs of eukaryotes and prokaryotes are homologous using this test. When applied to the comparison of the two known sequences of 5.8S RNA[5,6], an unequivocal relationship is apparent (see Table 1). It would seem, given the relatively large difference between the 5S sequences from yeast and humans[7,8], that 5.8S RNAs are more conservative to evolutionary change than eukaryotic 5S RNA. Such resistance to the fixation of natural mutations usually indicates an important structural and functional role. In fact, the sequences of all mammalian 5S RNA are apparently identical[9] and the same holds for 5.8S RNA[6]. The fact that both molecules are evolutionarily conservative increases the probability of finding a common origin, if in fact one exists

As shown in Table 1, the test when applied to the comparison of either the eukaryotic or prokaryotic 5S RNA sequence with either 5.8S RNA sequence (yeast and hepatoma) shows them to be no more related than random sequences.

One might argue that in spite of the conservative evolution of these molecules, all similarities of sequences have been wiped out during their independant evolution from a hypothetical ancestor. We have, however, developed methods to infer hypothetical ancestral sequences using known 5S RNA sequences[10]. With a corpus of 15 known sequences we have estimated ancestral prokaryote and eukaryote sequences: no 5S RNA ancestral sequences show any relationship to known 5.8S RNA sequences.

These results indicate that 5.8S RNA does not share a common ancestor with 5S RNA and therefore did not arise by duplication of the 5S RNA gene followed by divergence. One interesting possibility is that the 5.8S RNA evolved from the intercistronic region of the bacterial ribosomal genome.

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Received November 3, 1975; accepted January 8, 1976

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Bean halo-blight toxin

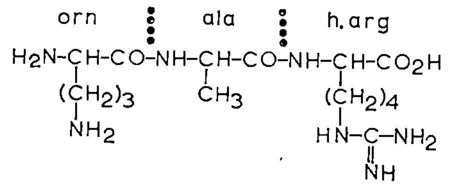
Pseudomonas phaseolicola causes a disease of beans commonly called halo blight. The halo is a chlorotic zone which appears in the leaf round each infection site. It has been interpreted as the effect of a toxin produced at, and diffusing away from, the infection site. The zone characteristically contains a relatively large amount of the amino acid ornithine[1]. When *Ps. phaseolicola* was grown in a defined liquid culture medium at 18 °C the filter-sterilised medium, when applied to bean leaves, caused the chlorosis symptom of the disease[2]. From such media I have isolated a substance, 80–90% pure, which, when introduced to the leaf at a level of 100 ng per g fresh weight, causes both chlorosis and accumulation of ornithine. I report here the highly unusual structure of this compound. Its extreme lability in the presence of weak acids has made its isolation difficult, but has helped in the resolution of its structure.

The pure substance is water soluble, and hydrolysis with 6 M HCl (100 °C, 18 h) liberates three amino acids (ornithine, alanine and homoarginine). In spite of the presence of two basic amino acids (ornithine and homoarginine) the toxin itself behaves during electrophoresis at *p*H 2 like the acidic amino acids, suggesting that it also contains strong acid groups. More gentle hydrolysis of the toxin with dilute HCl (0.01 M, 40 °C, 2 h) yields a new compound which contains the same amino acids as the toxin but which shows no toxic properties. This new compound has, at *p*H 2, an electrophoretic mobility a little greater than that of ornithine, and now consistent with its content of basic amino acids. Sequential cleavage with carboxypeptidase and gas chromatography–mass spectrometry showed it to be the tripeptide ornithylalanylhomoarginine (structure 1, Fig. 1).

Because ornithine is the N-terminal amino acid of the tripeptide, both ornithine amino groups are unbonded. When, however, the intact toxin was reacted with 1-fluoro-2,4-dinitrobenzene and the product was hydrolysed, N^α-dinitrophenylornithine was the only amino acid derivative produced. The tripeptide, in contrast, gave N^α,N^δ-di-dinitrophenylornithine. This shows that the δ-NH_2 of the toxin is bonded. There are two possible interpretations: either the toxin structure is cyclic (for example, cyclic-N^δ-ornithylalanylhomoarginyl) incorporating the ornithyl side chain in the ring (in which case dilute acid must open the ring at the ornithyl-N^δ-peptide bond), or the toxin structure is linear with a non-peptide group bonded to the ornithyl-N^δ (in which case dilute acid would yield peptide and non-peptide products; structure 2, Fig. 1).

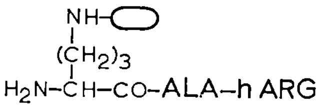
The former was excluded when the action of carboxypeptidase enzymes on the toxin was studied. Homoarginine, then alanine, were sequentially cleaved, yielding a product which contained only one amino acid, ornithine. Because carboxypeptidase requires the presence of a free terminal carboxylic acid group to be able to act on the adjacent peptide bond (hence its sequential action), the cyclic structure would not have been attacked by it. I therefore conclude that the toxin is represented by structure 2, and that there is a further group present that is liberated from the toxin by the action of dilute acid.

The product of the carboxypeptidase reaction, which is ornithine with some unknown group still attached, behaves as a toxin on bean leaves, and has unusual properties. At *p*H 2 ornithine alone migrates electrophoretically as a strong cation. The unknown compound, however, which contains ornithine, migrates as an anion, indicating that there must be a compensating strongly negative charge. Carboxylic acid groups are not sufficiently anionic at *p*H 2 to satisfy this structural requirement; biologically, the most likely groups to have the required properties are phosphate or sulphate. Specific detection procedures for phosphate[3] showed that the toxin and the carboxypeptidase products all contained phosphate. When the bacteria were grown in medium containing ^{32}P-phosphate, ^{32}P was incorporated into the toxin. One of the products of acid hydrolysis of the ^{32}P-toxin did not contain ^{32}P, and had the electrophoretic properties of inorganic sulphate. When the bacteria were grown in medium containing ^{35}S-sulphate, ^{35}S was also incorporated into the toxin. On acid hydrolysis of the toxin, ^{32}P and ^{35}S were released in PO_4^{3-} (P_i) and SO_4^{2-}, respectively.



$H_2N\text{-}CH[(CH_2)_3NH_2]\text{-}CO\text{-}NH\text{-}CH(CH_3)\text{-}CO\text{-}NH\text{-}CH[(CH_2)_4HN\text{-}C(=NH)\text{-}NH_2]\text{-}CO_2H$

1

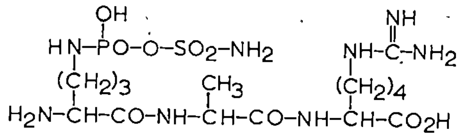


$H_2N\text{-}CH[(CH_2)_3NH\text{-}\bigcirc]\text{-}CO\text{-}ALA\text{-}hARG$

2

$HO\text{-}SO_2\text{-}NH_2$ 3

$(HO)_2PO\text{-}O\text{-}SO_2\text{-}NH_2$ 4



$H_2N\text{-}CH[(CH_2)_3HN\text{-}PO(OH)\text{-}O\text{-}SO_2\text{-}NH_2]\text{-}CO\text{-}NH\text{-}CH(CH_3)\text{-}CO\text{-}NH\text{-}CH[(CH_2)_4NH\text{-}C(=NH)\text{-}NH_2]\text{-}CO_2H$

5

Fig. 1 Proposed structures.

The time course of hydrolysis by dilute acid gave further information (Fig. 2). The toxin was broken down rapidly, yielding the tripeptide and an intermediate (I) which contained both P and S. This intermediate hydrolysed rather more slowly, yielding free P_i (P_i estimation showed that there was 1 mol per mol of toxin) and another compound (which contained no P) which I call intermediate II. Free inorganic sulphate was liberated from intermediate II under more vigorous conditions (0.6 M HCl, 60 °C).

I then investigated the identity of intermediate II. Yield estimates based on ^{35}S measurements indicated a molecular weight (as its ammonium salt) of about 120. Initial chromatographic studies indicated that it was similar to synthetic sulphate esters of low molecular weight hydroxyacids, for example glycolic acid-*O*-sulphate and lactic acid-*O*-sulphate. These compounds had characteristic proton magnetic resonance (PMR) spectra. Therefore, 40 mg of purified ^{35}S-toxin was hydrolysed gently to yield 6 mg of purified intermediate II for PMR study. After proton exchange in D_2O, the sample was thoroughly dried, and the PMR spectrum in 99.8% D_2O was examined. Unlike a similar quantity of lactic acid-*O*-sulphate, the sulphate compound from the toxin showed no proton signals (apart from HOD). The only likely compound to satisfy the requirement of low molecular weight and no non-exchangeable protons is sulphamic acid (molecular weight, ammonium sulphamate 114). Authentic sulphamic acid was indistinguishable chromatographically and electrophoretically from intermediate II; also both reacted with cold dilute nitrous acid to yield free inorganic sulphate.

The structural components of the toxin are now apparent and its final structure depends on establishing the way sulphamic acid, P_i and the tripeptide are linked together. Intermediate I, which contains both P and S (but not the peptide), reacted with cold dilute nitrous acid in the same way as sulphamic acid, yielding a new product still containing both phosphate and sulphate. This indicates that, like sulphamic acid, intermediate I contains a terminal NH_2. Thus, the phosphate group of intermediate I is connected to sulphamic acid through S, not N, and the structure of intermediate I is established as 4 in Fig. 1. The structure of the toxin is thus 5 in Fig. 1. As far as I can determine, this peptide-ornithyl-N^δ-phosphosulphamyl type of structure has no direct parallel in the chemistry of natural products.

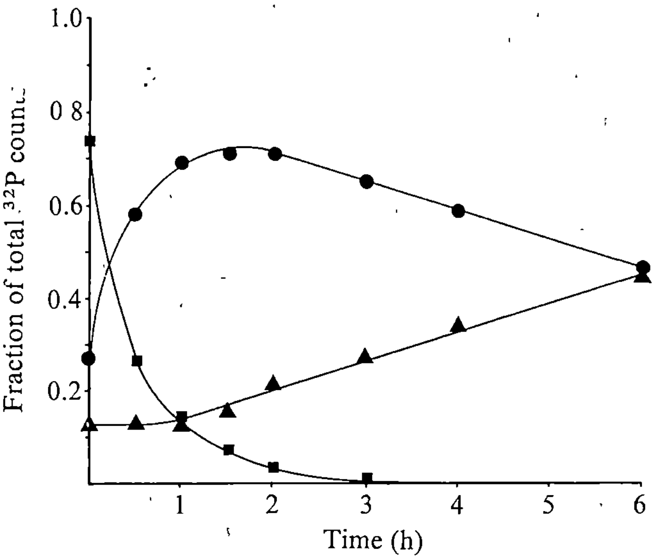


Fig. 2 Time course of hydrolysis of ^{32}P-toxin with 0.025 M HCl (40 °C). ●, Intermediate I; ▲, PO_4^{3-}; ■, toxin.

The action of the toxin in pathogenesis remains to be determined but it may be possible to account for accumulation of ornithine in the tissues of the diseased plant. An important biochemical reaction involving ornithine is its condensation with carbamyl phosphate to form citrulline. Two structural features of the toxin may be significant: the effective part of the toxin is a N^δ-substituted ornithine; and, the substituent group is an analogue of carbamyl phosphate, in which the >CO group is replaced by $>SO_2$. The toxin may therefore be useful in the study of the mechanism of action of ornithine transcarbamylase.

I thank Miss Beth Parsons for technical assistance, and Dr R. L. Bieleski for helpful discussions.

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Received December 15, 1975, accepted January 16, 1976.

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matters arising

Niche breadth in Bryozoans

O'Connor *et al.*[1] consider a resource gradient with an optimum point for a particular species. Other factors being equal, individuals migrating to a new environment will tend to select the optimum, or near optimum. As population density increases, the advantages of being near the optimum may be offset by intraspecific competition[2]. Migrants may therefore choose a suboptimal point on the resource gradient. As population density increases so will the range of resource gradient used.

O'Connor *et al.*[1] use the phenomenon of an increase in range with population density as a test for intraspecific competition. This is not a suitable test because range will increase with population density even in the absence of intraspecific competition. If intraspecific competition was not occurring and individuals chose the resource optimum with an associated variance from a normal distribution (say, mean of zero and variance of unity), then the expected range would increase with population density (going from five to fifty individuals the expected range would increase from 2.32 to 4.50 (ref 3)). It is not necessary to postulate a normal distribution or even a resource optimum for an expected increase of range with population density.

Using the epiphytic bryozoan *Alcyonidium hirsutum* settling on the serrated wrack *Fucus serratus*, it is probably permissible to replace the percentage cover used by O'Connor *et al*[1] by the numbers of individuals over a wide range of settlement density, provided that settled individuals are of the same age[4]. The optimum on the particular resource gradient used has been investigated previously[5].

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O'Connor et al. reply—We accept the point raised by Harvey *et al*[1] but it does not alter our earlier conclusion that *Alcyonidium hirsutum* shows increased niche breadth in the presence of intraspecific competition and decreased niche breadth in the presence of interspecific competition. Range on the resource gradient is indeed correlated statistically with the number of bryozoan settlements on each *Fucus serratus* frond, but because these fronds vary in age along their length, it is less reasonable to assume that the resulting colonies should be of equal age. In practice the partial correlation coefficient between *Alcyonidium* abundance (percentage cover) and its range, controlling for the number of faces colonised on each plant (and thus for the effect suggested by Harvey *et al.*) was 0.506 ($P<0.05$) That is, *Alcyonidium* shows increased range in resource utilisation as its own population levels rise, and does so independently of the statistical artefact suggested.

For the other three bryozoan species whose distribution we analysed, we reported small positive correlations between range and abundance, in small samples containing some unmeasured interspecific competition Correcting for the possible statistical effect by partial correlation left us with positive correlations in the case of *Flustrellidra hispida* ($r_p=0.419$, $P<0.2$) and *Electra pilosa* ($r_p=0.215$, $P<0.3$), but with a negative value in the case of *Membranipora membranacea* ($r_p=-0.463$, $P<0.2$). Thus only for this species might our first results have originated in a sample-size effect, though as we pointed out previously, we have available to us only samples which are subject to some degree of interspecific competition which tends to reduce niche breadth anyway In summary, therefore, our results still indicate that intraspecific and interspecific competition lead respectively to increase and decrease in niche breadth in *A. hirsutum* and possibly in other bryozoan species as well

We thank Alec Vardy for reviewing our statistical argument.

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[1] Harvey, P. H., Ryland, J S., and Hayward, P J, *Nature*, 260, 77 (1976).

The claim of O'Connor *et al.*[1] that distributional and density data for the bryozoan *Alcyonidium hirsutum* growing on *Fucus* fronds demonstrate the effects of both intra- and interspecific competition on niche width is unsound for both methodological and theoretical reasons. A positive correlation would be expected between the number of bryozoan colonies and the 'population level' as measured by percentage cover, and therefore the positive relationship found between the range of segments utilised and the population level is explicable without recourse to competition theory. On fronds which are more suitable overall, some of the less favoured segments would become acceptable, so that the frond is colonised more heavily and over a wider range of segments Other models will also produce this result. If darts are thrown at a linear target the range between darts should increase with the dart 'population size'.

Regarding interspecific competition we note that in the no-, one-, and two-competitor cases, decreasing sample sizes are used. A narrower niche width would thus be expected in the cases with competitors irrespective of interspecific effects. The utilisation curves for the first two cases, however, do not differ significantly (Kolmogorov–Smirnov two-sample test[2]), negating conclusions based on supposed differences between them. The two-competitor curve is based on too few colonised segments (18) for much confidence to be placed in supposed trends; furthermore, when allowance is made for the modal shift there is no significant difference between this and the non-competitor curve. Finally, B.

$$(\ln B = -\Sigma p_i \ln p_i)$$

is an accepted measure of niche width[3,4]. The value for the one-competitor case is higher than for the no-competitor curve, contrary to the prediction of O'Connor *et al.*[1].

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O'CONNOR ET AL REPLY—Crozier and Day[1] offer four distinct criticisms of our analysis[2] of niche breadth in Bryozoa. They suggest, first, that on fronds which are more suitable overall some of the less favoured segments would become acceptable, yielding a competition-free correlation between range and population level. As we pointed out explicitly in our paper, however, the available space at the optimum height is not physically filled at the higher population levels, so that animals settling elsewhere are behaving maladaptively if intraspecific competition is absent. Competition theory readily explains such suboptimal settlement in terms of a balance between the disadvantages of being away from the competition-free optimum of the resource gradient and the advantages of the reduced intraspecific competition found away from the optimum[3,4]; the model suggested by Crozier and Day[1] leaves us, on the other hand, with unexplained maladaptive behaviour on the part of the bryozoans.

The linear dart target criticism advanced is essentially that of Harvey *et al.*[5], to which we have responded with a partial correlation analysis[6] demonstrating that increase in resource range with increasing *Alcyonidium* population size was independent of the number of observations or 'darts'. We note in addition here that the dart effect is in any case too small to account for our results. Thus, if the change in *Alcyonidium* numbers between 2% and 4% cover (the latter being our median value) involved an increase of as few as 2 individuals at settlement, the dart effect predicts[7] only an increase in range of 84%, whereas we empirically observed an increase of 178%; for a slightly more realistic change of from 10 to 20 individuals, the dart effect is even less (21.4%), increasing the shortfall from the observed change even further. Similar calculations over other ranges of population size confirm our original conclusion, since the observed range increases are always substantially larger than any possible effect of sample size.

The third criticism by Crozier and Day[1], that the effect of sample size could explain our interspecific results, is incorrect on two grounds. First, for our interspecific analysis we used not the range of the resource utilisation function but rather its width at half height. This measure more closely resembles a standard deviation than a range in its statistical behaviour, that is, it is largely insensitive to changes in sample size. Second, even if this measure had been subject to the effect of sample size, the resulting increase would have been too small to account for the observed changes in niche breadth. Thus, for *Alcyonidium* our sample size effectively doubled (from 69 to 149) between the one-competitor and no-competitor situations: Fisher and Yates' table[7] shows that the effect of such a doubling of sample size would have been less than 14%, whereas the observed increase was of 34%, substantially larger. Similar calculations for the other seven comparisons show that in six cases the dart effect is too small by even bigger factors to account for the observed increases: only for the change in *Membranipora* niche breadth between the one- and two-competitor situations could an effect of sample size have accounted for the observed change. Therefore, even after assuming we had used range, and not the more stable width at half height, as our measure of niche breadth, the effects of the differences in sample sizes are so small that after making full allowance for them, seven of the eight comparisons are still indicative of competitive effects (Sign test[8], $P<0.05$), that is, the data still demonstrate that the presence of interspecific competitors on fronds reduces bryozoan niche breadths on those fronds We thus disregard this criticism of Crozier and Day[1].

Their final criticism is that the analyses chosen by them failed to support our conclusions. We note first that they overstate their case with respect to the Kolmogorov–Smirnov test: Siegel[8] explicitly states (p. 129) that the failure of this test to reject a null hypothesis (here that the one-competitor and no-competitor curves do not differ) does not mean that the hypothesis should then be accepted, contrary to the assertion of Crozier and Day. Second, we note that the formula

$$\ln B = -\Sigma p_i \ln p_i$$

requires that niche breadth be related to the Shannon–Wiener index of species diversity, H, by $B=\exp(H)$. Niche breadth and species diversity are thus mathematically related Since we know from empirical evidence[9] that different diversity indices lead to different biological conclusions, even when applied to the same data, we are unable to accept any one of the related niche breadth formulae as definitive The widespread acceptance of any particular formula may merely reflect uncritical acceptance of its hidden assumptions The measure of niche breadth we used has the merit of being distribution free and has an even more widespread acceptance in measuring distribution widths[10] than the information theory measure tested by Crozier and Day. We would naturally like to see all niche breadth measures tested with our data supporting our conclusions, but when simple measures support biologically sensible conclusions and complex measures fail to do so, we feel it appropriate to suggest that maybe the mathematics, rather than the biology, is at fault.

In summary, therefore, the criticisms offered by Crozier and Day[1] are only partially valid, and have too small an effect to invalidate our conclusions.

We thank Jim Gilliam for helpful discussion.

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Enteropancreatic circulation of digestive enzyme

WE are studying highly specific intracellular proteinases and have read with interest yet alarm the two recent reports[1,2] by Rothman *et al* proposing an enteropancreatic circulation for digestive enzymes. This seems unlikely for the following reasons (accepting the premise that protein molecules can be transported across intestinal and pancreatic acinar cells).

If the proteins are absorbed into the bloodstream in the active enzyme forms (chymotrypsin and trypsin, for example) they would be inactivated very rapidly by the plasma inhibitors such as α_1-antitrypsin, α_1-antichymotrypsin and α_2-macroglobulin before they could hydrolyse physiological substrates like prothrombin, fibrinogen, plasminogen and kininogen. If any active molecules were lucky enough to escape this bombardment by circulating inhibitors and be reabsorbed by the pancreas, what a cruel fate to be inactivated at the last hurdle by the pancreatic inhibitors.

If the proteins are absorbed in the non-activated forms of chymotrypsinogen and trypsinogen, they might just escape the attention of the inhibitors and be reabsorbed by the pancreas but to what avail? If an excess of "protein" is secreted into the intestine, Rothman *et al* seem to be proposing that a large percentage of this can be conserved by recycling. The activation processes for zymogens such as chymotrypsinogen and trypsinogen would, however, seem to be too rapid and complete to allow any significant level of zymogen to remain. The digestive capacity is controlled, not by the degree of zymogen activation in the intestine, but by the absolute amount of zymogens released on hormonal stimulus of the pancreas.

Space will not permit critical evaluation or alternative explanation for Rothman *et al.'s* observations Their experiments have been conducted by following "insensitive" parameters such as protein output by the pancreas and chasing a few radioactive counts around the bloodstream. It would be illuminating to see the experiment done wherein (potential) enzymic activity is followed. Only then would we have to consider the feasibility of an enteropancreatic circulation.

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ROTHMAN, GÖTZE AND LIEBOW REPLY—We are sorry that our observations and ideas have alarmed Kay and Beynon[1]; however, new scientific findings, are appropriately met with interest, not fear They feel that our observations must be explained in other, although unspecified, ways because in their view the circulation of digestive enzyme is quite impossible. If we understand their position correctly, they argue that all active proteolytic enzyme would be irreversibly bound in blood and that proenzyme entering the intestine would be immediately and completely activated in all circumstances and thus be unavailable for circulation. Since these postulates refer only to proteolytic enzyme and since a large percentage of the pancreatic digestive enzymes are not proteolytic, Kay and Beynon are apparently not questioning the existence of a circulation of digestive enzyme, but more narrowly the circulation of proteolytic digestive enzymes We have directed our response to this question.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Although the workings and physiological functions of plasma protein inhibitors are not completely understood. certainly the mere presence of various inhibitors in the blood (or acinar cell) does not mean that all enzyme is irreversibly bound by these molecules and taken out of circulation. On the contrary, it is the presence of these inhibitors in blood that makes it reasonable to conceive of active proteolytic enzyme being circulated. The point is that to prove whether or not active proteolytic digestive enzyme is circulated is a matter for further investigation, not presupposition. Relative to the potential for proenzyme circulation, Kay and Beynon do not cite the evidence on which they base their contention—that the activation of enzyme in the intestine is "too rapid and complete to allow any significant level of zymogen to remain." We are unaware of such evidence. They should remember that maximal activation does not mean that all the enzyme molecules have been activated, and that the activation kinetics of a few defined components in a test tube does not necessarily reflect what goes on in the intestine in various physiological situations. Kay and Beynon apparently insist on restricting the potential meaning of a biological phenomenon to their view of present knowledge of the chemistry and function of certain molecules. They thereby assume that they understand the chemistry and function of these molecules fully and that there are no unknown elements in the piece. On what basis do they make these assumptions?

Finally, in spite of their preconceived notions, the enteropancreatic circulation of digestive enzyme is a fact documented, not by "chasing a few counts around the bloodstream" as they egregiously suggest, but by demonstrating, with tracer kinetics, that labelled chymotrypsinogen instilled in the intestine reappears in pancreatic secretion collected directly from the duct and exclusively in the chymotrypsinogen band of secreted proteins separated by electrophoresis.

In the recent paper[2] which prompted Kay and Beynon's letter[1], we described experiments which suggest that this circulation may account for the movement of substantial amounts of protein. Two corollary observations further reinforce the interpretation that enzyme circulation is involved: (1) output (the increase in protein secretion) correlates strongly with input (digestive enzyme added to the intestinal lumen or injected into the bloodstream) with a slope of 0.92, or in other words, enhanced protein secretion by the pancreas accounted for 92% of protein input; and (2) these two variables fit the same regression line whether the collected secretion was injected into the bloodstream or instilled into the intestine. While these observations are subject to other interpretations (which were carefully pointed out in our article and which Kay and Beynon do not acknowledge in their letter), the circulation of substantial amounts of digestive enzyme is the most direct, and thus, the best interpretation of the results. It is on this basis that we feel it should be the hypothesis for the moment. If Kay and Beynon really have a more convincing interpretation of these experiments, then why do they hesitate to present it? We would greet it with interest, not alarm.

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Doubts about the role of the locus coeruleus in learning and the phosphorylation mechanism engaged in the cerebellum

GILBERT[1] has proposed a model of how the cerebellum, with the assistance of the locus coeruleus, could memorise movements. We have been studying the anatomy and chemistry of these and related systems, and have evidence that bears directly on that hypothesis.

The first point concerns the crucial role of the locus coeruleus in the memory consolidation of "motor signals stored in the cerebellum"[1]. The evidence to support that view comes from one study[2] which observed the effects of locus coeruleus lesions on learning. The results have, however, been brought into question[3]. Using a different approach, our laboratory has been studying the effects of localised brain stimulation on learning and retention performances (see ref. 4). Quite remarkable localisations have been obtained in the medial nucleus of the amygdala[5], substantia nigra, pars compacta[6], medial and sulcal portions of frontal cortex[7] and the lateral portions of the medial forebrain bundle[8]. In these specific regions, but not in surrounding locations, brain stimulation during learning disrupted retention. Using the same paradigm we have found no effect of locus coeruleus stimulation[7]. The results cast doubts on the essentiality of the locus coeruleus to the learning and memory of movements.

Related to the issue of the involvement of the locus coeruleus in learning is the point made by Gilbert[1] that "the locus coeruleus... will support intracranial self-stimulation". Though it is not to be denied that intracranial self-stimulation (ICSS) can be obtained when electrodes are in the region of the locus coeruleus[9], it is by no means proven that the locus coeruleus or its axonal system is responsible for that behaviour. Our evidence argues against a role for the locus coeruleus in intracranial self-stimulation Intracranial self-stimulation in the region of the dorsal bundle, the efferent ascending pathway of the locus coeruleus, is unaffected by the bilateral destruction of the locus coeruleus[10]: and intracranial self-stimulation has not always been obtained when electrodes are in the locus coeruleus[11,12] Given the uncertainty of the locus coeruleus involvement in brain stimulation reward it seems premature to invoke a role for it in memorising learned movements.

The second point concerns the mechanism of storage proposed by Gilbert[1]: the phosphorylation of membrane protein. We have studied the endogenous phosphorylation of four protein components of brain membranes[13], and have found that one component, which we term band F, is altered by a training experience[14]. This effect is most noticeable in the caudate nucleus, but alterations (admittedly of a less dramatic nature) have also been observed in the cerebal cortex. An analysis of the vermis of the cerebellum, however, has yielded negative results; that is, there is no change in cerebellar membrane phosphorylation as a consequence of training such as was observed with membranes of the caudate nucleus Although this represents an initial finding in two replicated experiments, the results gathered to date lend no support to Gilbert's views[1] on the location of the mechanism of storage. Actually, the phosphorylation mechanism itself may be appropriate, but its location may be elsewhere, for example, in the caudate nucleus, rather than in the cerebellum.

Though I am in sympathy with the approach offered by Gilbert, I question the detailed mechanism proposed, both with respect to the involvement of the locus coeruleus in learning and reinforcement and with respect to alterations in phosphorylation within the cerebellum. Our data, though negative on both specific points, point to particular brain locations potentially involved in memory, and to related regions where chemical storage mechanisms could be engaged following a learning experience.

I thank J. U. D. Sonratliff for technical assistance and David Morgan for helpful discussion. This work was supported by grants from the Alfred P. Sloane Foundation, NIH and NSF.

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DR GILBERT REPLIES—To support my proposal about motor learning in the cerebellum[1], I cited a study[2] which showed that locus coeruleus lesions markedly impaired, or abolished, the ability to learn to run with increased speed in an L-shaped runway for a food reward. Routtenberg questions the result of my study (see refs 1 and 2) because there seems to be no learning deficit in a T-maze discrimination task for animals with locus ceoruleus lesions[3]. The types of learning being tested in those studies are, however, quite different; and the results are not necessarily contradictory. I have described[1,4] how the cerebellum could store information required for the production of coordinated muscular activity in a learned movement. I have not suggested that the cerebellum is involved in other types of learning such as T-maze discrimination, and the fact that there is no learning deficit for this task with locus coeruleus lesions is irrelevant to my proposal.

Stimulation of the locus coeruleus probably leads to an increased release of noradrenaline at Purkyne cells[5] and could therefore lead to enhanced retention. The enhancement of retention by central administration of noradrenaline only occurs after depletion of central noradrenaline stores[6], however, and the stimulation of the locus coeruleus could be expected to enhance retention only in similar conditions. Therefore, the negative results of locus coeruleus stimulation[7] do not conflict with its proposed role[1] in long term memory consolidation.

Two independent studies[8,9] have shown that the locus coeruleus will support intracranial self-stimulation (ICSS), though with different experimental conditions no ICSS is obtained[10,11]. There seems to be no doubt that the locus coeruleus is closely involved with the positive reinforcement system of the brain, although I agree that the details of this involvement have not been determined. My theory does not require that the locus coeruleus cells directly contracting Purkyne cells support ICSS, but only that the locus coeruleus cells receive an input from the positive reinforcement system.

My suggestions about learning in the cerebellum were made on the basis of the large amount known about cerebellar anatomy and physiology. I would expect a similar memory mechanism to operate in other parts of the brain, however, especially in view of the widespread distribution of noradrenaline nerve terminals[12]. It is therefore very encouraging that a change in membrane phosphorylation as a consequence of training has been demonstrated[13] for certain regions of the brain. The possibility that this mechanism operates in the cerebellum has certainly not been excluded by preliminary experiments on a small number of protein components of membranes from a restricted region of the cerebellum.

In conclusion I am pleased that experiments are being carried out which test my proposed memory mechanism. I would add that there is increasing support[14,15] for the idea that the cerebellum is involved in motor learning.

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reviews

Renaissance and revolution

John Watling

The Nature of Scientific Discovery. (A Symposium Commemorating the 500th Anniversary of the Birth of Nicolaus Copernicus.) Edited by Owen Gingerich. Pp. 616. (Smithsonian Institution: Washington, DC, 1975.) $15.

Painting of Copernicus by Jean-Leon Huens

THIS misleadingly titled book is a record of a symposium held in 1973 by the Smithsonian Institute of the United States to commemorate the 500th anniversary of the birth of Copernicus. Besides the papers read at the symposium, the book includes a description of the proceedings, a large number of introductory speeches, photographs from a commemorative film made by Charles Eames, a black and white reproduction of a painting of Copernicus by Leonard Baskin and a verbatim report of the discussions which followed some of the papers. The proceedings included a performance of a musical work entitled "Copernicus—Narrative and Credo" by Leo Smit to a text written, and, in that performance, spoken, by Fred Hoyle. Neither text nor score are provided, but the text seems to have been an account of Copernicus's life and times. One of the speeches relates that Edward Rosen, who has translated some of Copernicus's major works, summed up the curiosity of Copernicus in the question "What is night, and how is it produced?". Such absurd exaggerations of the ignorance of Copernicus's contemporaries can only hinder an appreciation of his achievement. Aristotle, the prevailing authority of the time, argues for the spherical shape of the Earth from the curved edge of its shadow on the Moon during a lunar eclipse (*De Caelo*, Book II, Chapter 14).

The declared object of the symposium was to investigate the nature of scientific discovery, taking Copernicus's work as an outstanding example, rather than to clarify and evaluate that work. In fact, the papers divide into three groups, those on aspects of the history of Copernicus's times, those on the nature of scientific discovery and those providing brief expositions of questions in present-day cosmology. The first group forms the bulk of the book and contains the only papers of serious interest. The four papers on scientific discovery throw little light on it. Toulmin expresses his opinion that science is losing its vigour in fragmentation and technology; it should turn back to its traditional allies, cosmology and theology. Gerald Holton expounds Einstein's thoughts on scientific discovery without contributing much to the wide development and criticism which such views have received, for example from Karl Popper. Heisenberg finds the influence of tradition in research in the fact that, once one man has discovered a problem, others are attracted to the task of solving it.

Surely studies of Copernicus's own work would have provided a worthier commemoration? There is no lack of problems concerning it. Copernicus demonstrated the possibility of a radical change of viewpoint, a change which brought into prominence exactly those relationships (the relationships to the sun) which, according to Newton's gravitational theory, were of the greatest importance in explaining planetary movements. Why did Copernicus propose a change whose advantages, it seems, he could not guess at? A number of points, however, are made which illuminate Copernicus's work and its importance. Bronowski finds the inspiration for the change of viewpoint in Renaissance studies of perspective, for those studies seek rules for determining how objects in space appear to observers situated at different vantage points. Gingerich points out the importance of Copernicus's change of viewpoint for Newton's theory. Rupert Hall stresses the mechanical problems posed by the need to reconcile the behaviour of bodies at the surface of the Earth with the Earth's motion: "If Galileo converted mechanics into a branch of natural philosophy, it was Copernicus who demanded that this be done."

The contributors are scarcely in accord over the significance of Copernicus's thought. Hall, as we have just seen, finds its value in the stimulus it gave to the investigation of real problems in dynamics. Toulmin, on the other hand, finds it in "the claim that the human mind does have the capacity to develop consistent and comprehensive theories about the world in which we live, and can do so on a scale that goes far beyond the reach of our experience." Again, whereas Bronowski finds Copernicus to have been "a humanist gentleman", inspired by the Renaissance enthusiasm for classical philosophy and literature, Toulmin finds him to be the re-incarnation of the greek scientific spirit, that of accosting the most profound questions about the natural world. Bronowski and Toulmin differ, too, about the scientific value of humanism.

(continued from previous page)

Bronowski claims that the Renaissance study of ancient texts established standards of accuracy and exactness of inference which revealed new possibilities to science. Toulmin holds that humanism favoured a scepticism concerning human knowledge inimical to confident theorising about nature. The most fundamental disagreement, however, is that between Bronowski and Heiko Obermann. Bronowski writes, "Copernicus's attitude implies that the single truth exists in nature and as nature, and cannot be established or overturned by any authority other than the study of nature herself." Obermann does not deny that that was Copernicus's attitude; what he does deny is that it is right. "Astronomy," he asserts, "cannot reveal the 'true causes' of astral phenomena insofar as final causality lies beyond its purview." Again, "the goal of the natural sciences is validity in the sense of accuracy, whereas that of the humanities, particularly of philosophy and theology, is validity in the sense of truth." How philosophy and theology are to reveal the true causes of astral phenomena, Obermann does not proceed to explain.

The historical papers I have mentioned are interesting, as are some that I have not found room for; unfortunately they are outweighed by a mass of material which is of very limited interest. □

Perceptive handbook

Handbook of Perception. Volume 5: Seeing. Edited by Edward C. Carterette and Morton P. Friedman. Pp. xxii+527. (Academic: New York and London, September 1975.) $28.50; £13.70.

THIS volume, the fourth to be published in a set of ten, could readily stand alone as an advanced textbook of visual perception. The contributors are well known, the text is uniformly clear and intelligent, and the level of presentation is more even than in earlier volumes. The organisation is conventional. Le Grand opens, with an informed history of visual science and an introduction to radiometry and photometry. In a distinguished chapter on receptive fields, J. Robson surveys the neurophysiology of the visual system and offers a shrewd discussion of the widely-canvassed idea that the visual cortex acts as an analyser of spatial frequencies. Colour vision is covered by R. and K. De Valois and by R. Boynton; temporal factors by L. Ganz; spatial resolution and interaction by J. Thomas; the perception of patterns and objects by Dodwell; depth perception by W. Richards; and the perception of movement by R. Sekuler. In a final chapter on Vision and Art, M. Pirenne discusses the difference between viewing a painting and viewing a *trompe l'oeil*. Although the chapter headings are classical the fresh concepts of the seventies have a firm place in the text. If there are no traces of the grand schools that once dominated textbooks of perception and if most of the debates are now at a technical level, then that may be wholesome enough. Dodwell indeed argues here that the need for all-embracing theory of visual perception has passed and that the many different processes necessary for perception must be considered individually and in detail.

What the book lacks is an Editor and its weakness lies in the same absence of coordination that marked earlier volumes: there is clear internal evidence that the contributors have not read each other's chapters and indeed there is grave doubt as to whether anyone with editorial authority has read the entire text. Thus the concepts of Fourier Analysis are independently introduced by Robson, by Ganz and by Thomas, when what was needed was one separate account written by a single hand. It is extraordinary, even outrageous, that Boynton and De Valois have not been invited, or have not been pressed, to read each other's excellent chapters on colour vision. Both chapters, for example, discuss the colour-naming work of Boynton and the attempt by De Valois to relate Boynton's results to the electrophysiology of the primate; each refers to earlier papers by the other but there are no cross-references between the two chapters. When cross-references do occur they are often blind: thus Le Grand writes "Chemical structure of rhodopsin and of other visual pigments will be discussed by Robson, in Chapter 4 of this volume", but the reader will turn to Chapter 4 in vain. **J. D. Mollon**

Subunit interactions in enzymes

Subunit Enzymes: Biochemistry and Functions. (Enzymology: A Series of Textbooks and Monographs.) Edited by Kurt E. Ebner. Pp. x+332. (Dekker: New York, August 1975.) $24.50.

THE aim of this book is to examine by means of representative examples the different types of subunit interactions that may exist in proteins. The proteins chosen for detailed consideration are glutamine synthetase of *Escherichia coli*, UDP-galactose-4-epimerase, lactose synthetase, acetyl coenzyme A carboxylases, tryptophan synthetase, and the steroid carrier protein. Each is the subject of a chapter written by research workers who have made major contributions to the field. An introductory chapter, on the general structure and thermodynamics of multisubunit proteins and molecular models for cooperativity, also contains sections on haemoglobin, CTP synthetase and glyceraldehyde-3-phosphate dehydrogenases, but these are not sufficiently detailed nor up-to-date to be of value. Most of the other chapters are clear and comprehensive accounts of the relationship between structure and function, with emphasis on the significance of the multisubunit structures; some include unpublished work and addenda containing recently published findings.

The acetyl coenzyme A carboxylases of *E. coli* and avian liver are described in an excellent article by Lane, Polakis and Moss. These are examples of protomers composed of several different polypeptide chains which catalyse distinct partial reactions or, in the case of the animal enzyme, combine with the allosteric activator citrate and induce association of the protomer to form the active linear polymer. Tryptophan synthetase (Crawford) has an $\alpha_2\beta_2$ structure, and the two types of polypeptide chain again catalyse separate partial reactions; in the protomer their catalytic efficiency is increased and dissociation of the indole intermediate is prevented. Glutamine synthetase is subject to covalent modification by an elaborate enzyme cascade system, regulated by metabolites, and in the article by Ginsberg and Stadtman the interactions between adenylylation, essential divalent cations and allosteric effectors are discussed in detail. Less is known about interactions between the two subunits in the UDP galactose epimerase molecule, which contains a single NAD^+ molecule acting unusually as a prosthetic group rather than a coenzyme (Gabriel, Kalckar and Darrow). The function of α lactalbumin as a modifier protein that decreases the K_m of galactosyl transferase for glucose as acceptor, and its possible role in the control of lactose synthesis in the mammary gland, is discussed by Ebner and Magee. The squalene and sterol carrier protein is needed for maximal activity of several enzymes involved in cholesterol and bile acid and synthesis, and its probable function as a lipid-carrier in these pathways is described by Dempsey.

This book serves a useful purpose in bringing together diverse examples of subunit interactions in enzymes. The omission of aspartate transcarbamylase is surprising. **K. Dalziel**

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CALCUTTA

Cell membranes

Pathobiology of Cell Membranes. Vol. 1. Edited by Benjamin F. Trump and A. U. Arstila. Pp. xv+497. (Academic: New York and London, August 1975.) $36.50; £17.50.

THIS volume is the first of a projected "multivolume treatise designed for pathologists and cell biologists interested in the role of membrane alterations in disease processes". Each of eleven chapters was written by one or more experts, and each is followed by an editors' summary. The subject matter ranges from broad topics such as "Cell Membranes and Disease Processes" (B. F. Trump and A. U. Arstila) and "Alterations in Lysosomal Membranes as Related to Disease Processes" to more technical ones, such as "The Use of Circular Dichroism in the Study of the Structure of Cell Membranes" (R. W. Henkens). In large measure, the book deals with the pathogenesis of subcellular lesions with special emphasis on the possible or probable roles of various cell membranes. Consideration is thus given to "The Cellular Mechanisms of Hormonally Induced Tissue Atrophy" (H. J. Helminen), "Thyroid Lysosomes in Health and Disease" (R. Seljelid), "Toxic Changes in Mitochondrial Membranes and Mitochondrial Function" (R. A. Goyer and B. C. Rhyne) and "Human Intestinal Epithelium as a Biological Membrane" (W. O. Dobbins III). These chapters contain much information of a correlational nature, particularly on the ultrastructural level. Other chapters give overviews of some key properties of cell membranes from physicochemical, biochemical, physiological and pathophysiological perspectives and include, in addition to the chapter on circular dichroism, the following topics: "Lipid Peroxidation and Fluorescent Molecular Damage to Membranes" (A. L. Tappel), "Colloid Osmotic Pressure as a Cause of Pathological Swelling of Cells" (J. R. Robinson), "Photopathology of the Erythrocyte Membrane" (J. S. Cook), "The Endocytic Uptake of Macromolecules" (P. J. Jacques).

All of the authors have made it clear that there are gaps between more or less defined phenomena in model membrane systems and the pathophysiological behaviour of cell membranes *in vivo.* Nowhere in biology have models been more popular than in the study of cell membranes. The search for pathogenetic mechanisms has of necessity been either empirical or reduced to narrowly defined, idealised situations. Aware of this, the editors have taken a comprehensive stance in their preface, choice of topics and comments, thus reflecting the Hegelian dictum "*das Wahre ist das Ganze.*"

The book has been written and edited with care, is beautifully illustrated and well printed. It represents a major effort to relate data and ideas about cellular membranes derived from model systems to pathobiological phenomena. It should be read by students of disease, not only to obtain information or as a guide to primary sources, but as an aid to planning experiments. I look forward to the publication of the subsequent volumes.

G. W. Richter

Microprobe analysis

Electron Microprobe Analysis. (Cambridge Monograph on Physics.) By S. J. B. Reed. Pp. xvi+400. (Cambridge University: London and New York, 1975.) £12; $34.50.

THIS is a very useful monograph that fills a timely need for anyone who is considering the possibility of entering the field of microprobe analysis. It is but a short mental step from knowing that high energy electrons produce X rays when they strike solid objects to deciding that this would be a good method of analysing the composition of the object. The pitfalls, however, are numerous. Since the germinal work of Castaing in 1951 the field has developed at an astounding rate, and it is now known how to design suitable instruments and how to convert raw data into compositional details. Instruments are complicated and very expensive, and the investigator must often make the kinds of decisions as when he buys an automobile —which of the optional 'extras' do I really need? The analysis of raw data is equally, if not more, complex as numerous (and not small) corrections must be applied.

Clearly a readable text was badly needed to guide both neophyte and expert through this mass of detail, and this book serves that purpose well. An expert would surely conclude that each individual chapter is too short and glosses over the interesting details, but the essential facts are presented in each case even though some chapters are condensed almost to the point of reading like a telegram. To cover the fields of instrument design and the meaning of the data, the author was forced to summarise a vast literature, and he is to be commended for his efforts.

It would, however, have been valuable to provide the reader with a list of applicable texts and monographs at the end of each chapter for further reading and more detailed study. A carefully selected list would have been more valuable than chasing down the many references scattered throughout the text.

A. V. Crewe

NEW

The Nature of Seawater

Edward D. Goldberg, Editor
Scripps Institution of Oceanography

Report of the Dahlem Workshop on
The Nature of Seawater
Berlin 1975, March 10 to 15

From the Introduction by E. D. Goldberg: Herein are an unusual set of presentations. In the main they were written by some of the world's most distinguished chemists, who describe the areas of research that they are pursuing. These articles in pure chemistry were prepared for a group of marine chemists who assembled in March 1975 in Berlin, Germany to consider new approaches which might yield a better understanding of the compositions and reactions of oceanic systems. Now they are being presented to a wider audience of marine chemists with the same aim in mind.

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obituary

Robert Stoneley sometimes remarked how much he regretted never having been a research student. The First World War took him away from University, and he returned to begin teaching straight away. Yet he made valuable contributions to theoretical geophysics during the years when he was heavily engaged in lecturing, first at Sheffield then at Leeds. His published papers were short and not frequent, averaging one a year, yet he made key contributions. His first paper, in 1924, investigated the possibility of waves channelled along the welded interface of two elastic media. These came to be called Stoneley waves, though he could never bring himself to use the name and spoke of 'interface waves of Rayleigh type'.

In 1925 another important paper showed that the observations of surface waves which had been used by seismologists to calculate phase velocities in fact gave group velocities. In 1926 he made a numerical study of the Earth's elastic yielding, using C. G. Knott's velocities of sound waves and shear waves throughout the Earth. This was one of the pioneer papers that led to the conclusion that the core of the Earth is liquid.

In 1931 Stoneley explored the significance of Jeffrey's remark that a deep earthquake should excite relatively small surface waves. This consequence of dynamical reciprocity was skilfully used by Stoneley to confirm that some earthquakes, suspect on account of anomalous travel times, had indeed originated at depths of several hundred kilometres.

During the 1920s and 1930s Stoneley worked both on theoretical problems of surface wave propagation in layered media, and on data analysis from seismograms or from tables in the International Seismological Summary. He showed how to separate double earthquakes, and how to distinguish in seismograms between Rayleigh and Love waves. Later he suggested to Michael Longuet-Higgins the work which led to an explanation of the origin of microseisms.

The Second World War, during which he carried very heavy teaching loads in Cambridge, interrupted his research for ten years. When he began to publish again it was in a valuable series of summaries or review articles on microseisms, waves in anisotropic media, surface waves, tsunami, oscillations of the Earth, deep structure of the Earth, and the history of modern seismology. He retained robust health and remarkable vigour for many years beyond retirement. During the period 1961–68 he served in Washington as consultant in the US Coast and Geodetic Survey and as Professor at the University of Pittsburgh.

A great formative influence in Robert Stoneley's career was his close friendship with Harold Jeffreys. Again and again in his papers there is reference to help or criticism from Jeffreys. Men of very different characters but similar in scientific interests—extending from differential equations to botany—their association was highly creative.

Robert Stoneley was a man of great personal kindness, responding eagerly to any request for help and going to any amount of trouble to provide it. He was patient and equitable, yet at the same time efficient, and the long list of organisations in which he served as secretary or chairman testifies to the confidence people placed in him. His advice and warm humanity were deeply appreciated everywhere.

E. R. Lapwood

George Hoyt Whipple, whose work in the 1920s led to the control of pernicious anaemia, died on February 1 at the age of 97.

After gaining his medical degree, at Johns Hopkins, Dr Whipple remained there until, at the age of 36, he became director of the Hooper foundation for medical research at the University of California. Whilst there he demonstrated that a diet containing raw liver was an effective way of treating experimental anaemia in dogs. That discovery led to clinical trials of raw liver for various anaemias culminating in 1926 when G. R. Minot and W. P. Murphy in Boston showed that pernicious anaemia, previously incurable, could be controlled by dietary liver. Dr Whipple's contribution to that success was recognised in his sharing the 1934 Nobel prize for medicine with Minot and Murphy. It was another fourteen years before teams at Merck and Glaxo simultaneously identified vitamin B_{12}, the active factor in the liver.

In 1921 Dr Whipple moved to Rochester University where he headed the newly founded medical school. During the next thirty years, first as professor of pathology and then as dean of the medical school, he continued to study the factors, particularly iron, that can reverse experimental anaemia. He also served as a trustee of the Rockefeller Foundation from 1927 to 1943 and as a scientific director from 1936 until 1953.

announcements

International meetings

April 15, Deadline for abstracts for **Nonspecific Immune Stimulation in Cancer and Autoimmune Disease** (to be held July 5–6 in Bucharest) (2nd International Symposium on Cancer Immunotherapy, Oncological Institute, P.O. Box 5916, Bucharest 12, Romania).

April 28–30, **Land as a Waste Management Alternative,** Rochester, N.Y. (Agricultural Waste Management Conference, 207 Riley-Robb Hall, Cornell University, Ithaca, New York 14583).

Person to Person

Going on sabattical with family to King's College, London, and would like to exchange a large 4-bedroom, furnished house in desirable area of Washington, D.C. and 20 min from NIH for comparable house or flat in interesting section of downtown London, with good schools. Seven months from June, but flexible. (Dr Mark Smulson, Department of Biochemistry, Georgetown University, Schools of Medicine and Dentistry, Washington, D.C. 20007.)

May 12–15, **Dictyostelium,** a workshop at Cold Spring Harbor, New York (Dr Harvey Lodish. Department of Biology, MIT, Cambridge, Mass, or Dr William Loomis, Department of Biology, University of California at San Diego, La Jolla, CA 92093).

May 30, Deadline for abstracts for the **27th Annual Session of the American Association for Laboratory Science** (to be held on November 7–12 at Houston) (Dr Charles W. McPherson, Animal Resources Branch, DRR, NIH, Bethesda MD20014).

nature

March 11, 1976

Inequality of opportunity: questions but no answers

THE table shows admissions to UK universities in October 1975 for a selection of subjects. An attempt is made not only to show how many places are available as a percentage of the number of candidates, but also to indicate how some subjects are major exporters, others major importers of students. The medical sciences continue to export, notably to biology and biochemistry, even though the numbers of applicants for medicine was sharply down. The vigour of biology is remarkable. Even though it imported 40% of its students, applicants who named biology as their first preference grew by 20% from the previous year. Biomedically inclined schoolchildren are probably being increasingly advised to apply for biology rather than medicine. The physical sciences continue in the doldrums.

However much a popular subject, such as medicine or veterinary science, may export, it is abundantly clear that many students with quite good qualifications are being turned away who would have been accepted with open arms if they had possessed the same qualifications in less popular subjects. Can we afford to do this? How could it be stopped?

Subject	Candidates preferring this subject (a)	Places filled in this subject ($b+d$)	Percentage imported $100d/(b+d)$	Percentage exported $100c/b$	Percentage of candidates who get in somewhere $100(b+c)/a$	Places per candidate $(b+d)/a$
Veterinary science	1,467*	278†	1	136	44	0.19
Medicine	12,046††	3,208	1	67	44	0.27
Architecture	2,345	640	3	25	33	0.27
Dentistry	2,969†	898	14	45	38	0.30
Pharmacy	2,679	818†	14	41	37	0.30
Law	8,351	3,200	4	25	46	0.38
Civil engineering	4,818*	2,422	13	14	50	0.50
Geography	4,128	2,098	9	18	54	0.51
Psychology	2,570	1,337†	17	19	51	0.52
Mechanical engineering	3,350*	1,789	17	12	50	0.53
Music	1,109**	609	3	4	55	0.55
English	5,666	3,177*	12	25	62	0.56
Electrical engineering	4,392	2,537	13	10	55	0.58
Sociology	2,820†	1,679	29	24	53	0.59
French	1,777†	1,217††	25	26	64	0.68
Biology	2,461****	1,714*	41	36	56	0.70
History	3,805	2,572	13	18	69	0.71
Economics	3,107**	2,233**	37	30	59	0.72
Mathematics	4,595†	3,395††	10	8	71	0.74
Physics	2,295††	1,990††	22	12	76	0.87
Classics	517	485†	12	5	87	0.94
Chemistry	2,292	2,211	30	7	73	0.96
Biochemistry	1,107	1,076†	52	25	58	0.98
ALL SUBJECTS (many not listed above)	131,478	71,211	—	—	54	0.54

a = No. of candidates preferring this subject.
b = No. of candidates preferring this subject and accepted for it.
c = No. of candidates preferring this subject and accepted for another.
d = No. of candidates preferring another subject and accepted for this one.
Each * represents a 5% increase in numbers over 1974 (the global upward trend having been removed).
Each † represents a 5% decrease in numbers over 1974 (the global upward trend having been removed)

SOURCE: Thirteenth Report of Universities Central Council on Admissions (UCCA).

Controlling the new proliferation

Trade in nuclear technology is increasing. **John Stares** considers whether the nuclear Non-Proliferation Treaty, six years old last week, can still effectively control the spread of nuclear weapons.

DURING the six years since the Treaty on the Non-Proliferation of Nuclear Weapons (NPT) came into force, the peaceful uses of nuclear technology have become much more widespread. With this development has come the increased risk of the misuse of nuclear technology, for military rather than peaceful purposes. Through peaceful nuclear programmes, many countries (in addition to the five nuclear-weapon powers) have accumulated the technical expertise and knowledge, as well as the fissile materials, necessary to develop and manufacture nuclear weapons. In time the number of such countries will grow, and some disarmament experts have predicted that within a few years more countries will in fact "go nuclear". The Indian nuclear test in the spring of 1974 clearly demonstrated that, even for an underdeveloped country, the technological and economic constraints on producing nuclear explosives (whether for peaceful or military ends) that existed a few years have largely disappeared. Is it possible, then, to prevent nuclear-weapon proliferation? If so, is the NPT an effective means of doing so? Or must other means be found?

It is certain that the global demand for energy will continue to grow, and that the rate of growth will be highest in the underdeveloped countries. Development requires a plentiful supply of energy—cheap energy—and in the face of shortages and rising prices of other energy sources (oil, for example) the only viable alternative energy source at the moment for many, if not most, underdeveloped countries is nuclear energy. The capital outlays for constructing nuclear power stations may be higher than for oil- or coal-fired power stations, but nuclear fuelling costs are considerably lower than conventional ones, thus making nuclear electricity generation at least competitive in terms of cost with other methods. This is well illustrated by the fact that Iran plans to produce much of its future energy from nuclear power stations, even though it has enormous oil reserves: it is more economical for Iran to sell or conserve its oil than to burn it in power stations.

It is therefore right and proper that nuclear energy should be made available to the underdeveloped countries. This was recognised in the NPT: the Preamble declares that "the benefits of peaceful applications of nuclear technology should be made available for peaceful purposes to all Parties to the Treaty". But any country with its own nuclear power reactor has, on its territory, the raw materials for making nuclear weapons. (It is interesting to note that if a power reactor is operated to produce the cheapest electricity, the plutonium it produces will consist of a mixture of isotopes and will be suitable for making only crude nuclear weapons, not optimally efficient ones.) The number of countries with nuclear programmes is increasing. In 1970 when the NPT became effective, 15 countries were operating power reactors. By 1975 the number had risen to 19. And if present plans are fulfilled, nearly 30 countries will have their own nuclear power stations by 1980. The increase in the number of actual reactors is even more dramatic. In 1975 about 170 power reactors were in operation, about two-thirds of them in the nuclear-weapon countries. By 1980 it is estimated that the total will have increased to more than 400, and that more than half will be in countries that do not, now, have nuclear weapons.

In theory, the NPT seemed a reasonable system for ensuring that the spread of peaceful nuclear technology did not lead to the proliferation of nuclear weapons. Under the Treaty, all non-nuclear-weapon countries undertook not to manufacture or otherwise acquire nuclear weapons, or indeed any nuclear explosive device, and to permit the application of control procedures, called safeguards, on its nuclear activities "with a view to preventing the diversion of nuclear energy from peaceful purposes to nuclear weapons or other nuclear explosive devices". All parties to the Treaty undertook not to supply fissile material or equipment designed for processing, use or production of fissile material to a non-nuclear-weapon country without safeguards being applied. The Treaty certainly has its weaknesses, perhaps the major one being that it is imbalanced in favour of the nuclear-weapon countries. These were left free to develop and increase their own nuclear arsenals, and were not obliged to submit to safeguards on their nuclear activities.

The Treaty therefore does not ensure the non-proliferation of nuclear weapons, but only seeks to stop their spread to non-nuclear-weapon countries. However, if *all* countries had joined the NPT, the nuclear proliferation problem might now be much less serious than it is: an international system of safeguards covering all nuclear activities (except in the nuclear-weapon countries) would be in operation, and all but five countries would have given up the option of producing nuclear weapons. But several important countries are still not members. This group includes two nuclear-weapon powers, France and China; several other industrial countries, such as Japan (which signed the Treaty in February 1970 but has still not ratified it); and many underdeveloped countries, India being the classic example and others including Brazil, Egypt (which has signed, but not ratified), Israel, Pakistan and South Africa. Many of the countries, industrial and underdeveloped, that remain outside the Treaty are already sufficiently advanced technologically to be able to produce their own nuclear weapons very quickly if ever they decide to do so. It is even believed that some may secretly have done so.

Can anyone prevent this . . .

Photo: Col

The nuclear exporting countries are clearly worried by this situation and by the possibility that future exports of nuclear materials and equipment might be diverted to military uses. There have been protests at nuclear exports in a number of countries: for example, in the United States there were calls during recent hearings of the Senate Government Operations Committee for an embargo on nuclear exports, and in the

Canadian Parliament the Opposition has criticised the Government for allowing economic interests to take precedence over more serious concerns about proliferation. Despite these protests, it is unlikely, for economic reasons, that exports will stop. The nuclear-weapon countries have invested huge sums in developing nuclear technology, and they and other industrial countries such as Japan and West Germany look to exports to boost their economies. The United States is reported to earn about $1 billion per year from its nuclear shipments; the West German deal to supply Brazil with a complete fuel cycle is worth some $5 billion; and the recently announced deal between Canada and South Korea for the supply of a 600 MW reactor involves Canadian, British and US loans totalling $400 million.

Concern over misuse

But although the nuclear exporting countries may be unwilling to halt exports, they are concerned to prevent the misuse of exported technology. During 1975, seven of them—the so-called Nuclear Exporters Group consisting of Canada, France, West Germany, Japan, the USSR, the UK and the USA—held a series of secret meetings on this issue. In January this year they were reported to have reached agreement on new safeguards and control procedures. The exact details remain secret, but according to Dr Fred Ikle, Director of the US Arms Control and Disarmament Agency, in testimony to the Senate Foreign Relations Subcommittee on Arms Control, it is thought that in future, requirements for nuclear exports will involve: acceptance by recipient countries of internationally accepted safeguards drawn up by the International Atomic Energy Agency; assurances by recipient countries that they will not use nuclear exports to make nuclear explosives, for any purpose; provision by importers of adequate security measures to prevent theft or sabotage; and assurances by importers not to transfer materials or duplicates of equipment to third parties without setting similar conditions.

The effect of such measures is not likely to be striking. They may be seen as an attempt to strengthen the NPT, since they are, in essence, similar to the provisions contained in the Treaty itself. In the past, disarmament experts have suggested that membership of the NPT should be demanded as a requirement for receiving nuclear assistance. Apart from the fact that some countries may regard such a move as even more restrictive and discriminatory than the NPT itself, the Group could hardly adopt such a policy when two of its members have not themselves joined the Treaty. The measures could thus be regarded as a "second best" answer to this suggestion. On the other hand, it is even possible to envisage that these measures might weaken, rather than strengthen, the NPT: countries that have not already joined the Treaty may now perceive that there is no pressing need to do so, since they know that the exporting countries are willing to supply assistance under terms that are similar to those of the NPT—but with the important difference that recipient countries will only be required to make a "gentleman's agreement" not to make nuclear weapons, which is very different from undertaking a formal international treaty obligation not to do so.

What must be accepted sooner or later is that it is simply not possible to *prevent* a country from acquiring the *potential* to produce nuclear weapons. Applying stringent controls or conditions may slow the process, but any country with a peaceful nuclear programme will inevitably acquire a nuclear-weapon potential in the end. What may be possible—in fact what may be the only answer—is to *discourage* countries from realising this potential. The only barrier left to the acquisition of a nuclear force, now that the technological and economic constraints have lost their importance, is a political one. For countries that have joined the NPT, this Treaty represents the barrier: to produce nuclear weapons would be a violation of international commitments. Once countries that have not joined the Treaty acquire a nuclear-weapon potential, they will have the choice whether or not to realise it. This choice will be influenced by political, not technological, considerations, principally the countries' perceptions of their security needs.

The key here is the attitude and behaviour of the nuclear-weapon powers themselves. If they indicate that they do not regard nuclear weapons as essential to security by showing themselves willing to make meaningful reductions in their nuclear arsenals, other countries might well be encouraged to follow their example, and renounce the nuclear option. In Article VI of the NPT, all parties undertook "to pursue negotiations in good faith on effective measures relating to the cessation of the nuclear arms race at an early date and to nuclear disarmament, and on a treaty on general and complete disarmament under strict and effective international control". It can hardly be said that the nuclear weapon powers have lived up to this commitment: the United States and the Soviet Union have been engaged in the Strategic Arms Limitation Talks (SALT) for years, but they have reached no agreements of any significant disarmament value, and the build-up of their nuclear arsenals continues unabated; Britain has also continued to develop its nuclear force, as have France and China, which have not even joined the NPT. An act of faith on the part of these powers, a demonstration that they are serious in their intention to abide by their treaty obligations and in their attempts to achieve nuclear disarmament, would have vastly more impact on world peace and security than any number of secret meetings. □

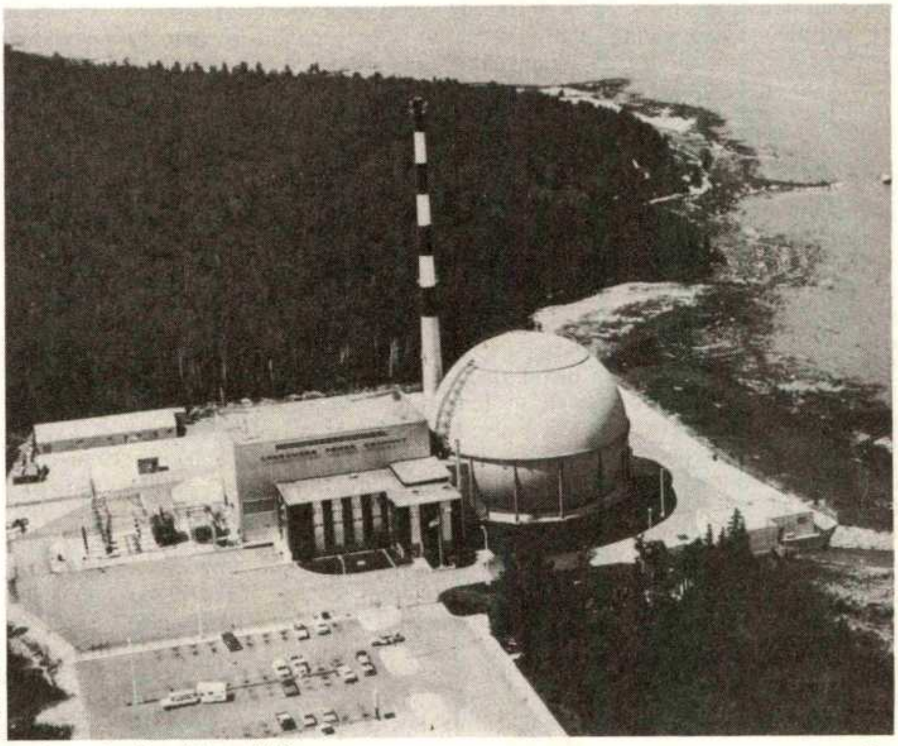

Photo: AEC

. . . being developed from this?

SPACE

Bringing cooperation nearer

A joint move backing planned European involvement with a major NASA space science project of the 1980s has come from the European Science Foundation and the US National Academy of Sciences. Allan Piper reports

FOLLOWING a meeting between the European Science Foundation and the US National Academy of Sciences at Williamsburg in Virginia, it has become clear that the independent scientific community represented by the two organisations means to become involved in the new $350-millions Large Space Telescope project (LST) planned for the 1980s by NASA. Recommendations developed during the meeting particularly underline European interest in the telescope, already under consideration as a priority project in the European Space Agency's programme of cooperation with NASA.

The LST, planned as a 2-metre diffraction limited optical telescope, should be in orbit by the end of 1983 as part of NASA's forthcoming space shuttle programme. Essentially an advanced and updated version of the highly successful Copernicus telescope launched four years ago, it will be used in planetary, galactic and intergalactic studies. The planned payload includes instrumentation covering the entire optical spectrum from infrared to ultraviolet, providing astronomers with a first opportunity to obtain data which is not accessible from Earth-bound observatories.

Described at Williamsburg as the single most important next step that could be taken in astronomy, the project hogged the spotlight during the wider debate on international cooperation on space observatories.

Recommendations resulting from the meeting call among other things for an independent, international LST Science Institute. An ESF-USNAS advisory committee is also suggested to oversee the selection of instruments for the telescope's scientific payload.

But while the recommendations outwardly stress the importance of independent scientific involvement, it is the implicit backing for ESA-NASA cooperation on the project which is more significant. None of the Williamsburg scientists was directly involved with the LST, but their statements, both space agencies have indicated, will weigh in decisions on the next moves.

Under the circumstances that could prove crucial. The project is running through heavy weather in the US, where Congress has just declined to approve NASA funding for 1977 in the absence of outside backing. The congressional coyness is seen as nothing more than a firm come-on to ESA, known to be close to throwing in its lot for a share of the price tag, but there is a very real danger that it will serve only to turn away a possibly faint-hearted suitor.

The problem is that Congressional and ESA decision-making are almost exactly six months out of phase. So even though there is confidence that Congress will give NASA the nod for 1978 funds if ESA adopts the project in the meantime, ESA officials who were in any case sceptical of the project's running power in competition with the other five possible cooperative space ventures will now have to be convinced additionally that it is not a complete non-starter.

Before losing heart, however, ESA can reflect that Congress may view the project more favourably once the present heavy expenditure on the space shuttle begins to run down. Moreover, there is still a glimmer of hope that the decision not to programme 1977 funds for the LST may be reversed. In the US the Congressional snub has brought a deluge of angry protest letters from astronomers and other interested scientists. There is little chance at this stage of additional funds, but Congress is holding open an option on internal reallocation.

Although the European astronomy community is not as well organised at an informal level as its American counterpart, the need for a tightly orchestrated campaign before, and not after, an ESA decision is widely recognised. Already there is European concern that ESA's option on the project will go by the board because of misguided views that it will not serve any immediate needs in astronomy over and above those provided by projects already in the pipeline, such as the Northern Hemisphere Observatory. It is at that level that the ESF Space Science Committee, which shares observers with ESA, will be most effective in pointing out that the LST offers new and unique possibilities, and is not just a superfluous bonus. ☐

COMECON

THE new Five Year Plans of the Comecon member countries began this year, and according to the Comecon Committee on Scientific and Technical Cooperation, which met in Budapest in January, scientific cooperation will be largely devoted to research projects and problems "which cannot be solved by individual countries". These include power production, protection of metals against corrosion, increase of protein production, the comprehensive utilisation of timber, and the testing of new insecticides. Discussions will also take place on the establishment of an integrated system for the exchange of scientific and technical information which, if successfully implemented, could eliminate uneconomical duplication of research.

Special emphasis is to be placed on research into energy resources ranging from lignite to nuclear power. The production and consumption of energy by the Comecon bloc is still steadily expanding: according to Nikolai Faddeev (USSR), the Secretary of Comecon, the nuclear power stations in the USSR and those built with Soviet assistance in Bulgaria, the GDR and Czechoslovakia now have an aggregate capacity of 7,500 MW, and by 1980 this figure will have risen to some 30,000 MW.

A 2,750 km pipeline from Orenburg to the western frontier of the Soviet Union will carry some 15,000 million m^3 of gas per year from the Orenburg gas condensate deposit to Bulgaria, Hungary, the GDR, Romania and Czechoslovakia. The second string of the *Druzhba* oil pipeline from the Soviet Union has been completed, and in 1975 delivered some 50 million tons of oil. Joseph Zamparo, of the University of Genoa, addressing the Conference on Energy Alternatives sponsored by the American Nuclear Society, said that energy consumption of the Communist countries (including China) was increasing by an average of 6.5%, whereas all other countries were reducing their energy consumption by amounts ranging from 9% for Western Europe to 2% for Australia.

USA

A $20–million boost for earthquake prediction studies hangs in the balance at the moment, following reluctance on the part of the Office of Management and Budget to sanction the extra spending. Much of the initiative in seeking out the extra money has come from Vice-President Nelson Rockefeller, who has been particularly active in support of extra funds for earthquake research since the disaster in Guatemala.

All is not lost, however, as proponents of earthquake research were testifying before a Congressional committee last week and pointing out, in particular, implications of the recent finding that a small part of California has uplifted very recently, by as much as 25cm in 10 years.

If the increase of $20 million were to be given the go-ahead, it seems likely that it would be spent not on the employment of additional personnel by the US Geological Survey but on research in private industry and universities. The programme would include work on the socio-economic aspects of earthquakes and their prediction, on the effects of earthquakes and on an effort to develop within a decade the capability to predict earthquakes satisfactorily.

The scheme is, however, "comatose" at present, though with luck and some judicious Congressional lobbying it may yet get off the ground.

● More evidence of the "drug lag" in the United States—whereby the Food and Drug Administration now takes so long to give permission for clinical trials and ultimately for the sale of a drug that some key preparations are available in the USA long after they go on the market elsewhere in the world—is contained in a recent report showing how and where the US pharmaceutical industry spent its money on research and development.

Figures released by the Pharmaceutical Manufacturers Association (PMA) recently indicate that of the $859 million spent by US drug companies on research and development in 1974, $133 million was spent outside the USA. The latter amount is 22% higher than the figure for 1973, which in turn was 66% higher than for 1972. The amount spent within the United States was 12.8% higher in 1974, at $726 million. The increases for 1975, as budgeted, were more modest, 12% for spending outside the United States and 10% for spending within the country.

The PMA points out that, on average, it now costs $11.5 million to develop a drug inside the USA and $7.5 million outside, and that whereas US companies had accounted for some 70% of the new chemical agents put on the market between 1940 and 1970, that proportion divided to some 39% between 1971 and 1973.

The PMA emphasises that it does not want the drug regulations to be relaxed, but simply to see the FDA become more efficient and responsive to the need to get new drugs on to the market as quickly as possible. In 1972 it took between 5½ and 8 years to get a drug passed by the FDA, by contrast with the situation elsewhere in the world where the comparable period of time, the association maintains, was 6 to 24 months.

Photo : Cam & Pen Int

Perhaps Senator Edward Kennedy's proposal to split up the FDA into two separate agencies and streamline the procedures for drug approval would help matters.

● The Nuclear Regulatory Commission (NRC) has been served up with a critical but constructive report by the Special Review Group it set up to look into the celebrated fire at Browns Ferry nuclear power station, Alabama, last year. The fire was started by a candle being used to check for air leaks around cables passing from one of the two reactor buildings to a cable spreading room beneath the control room.

The report points out that the inflammable polyurethane foam used to block off air draughts was not in the original design for the plant, and, like the candle, should not have been used. The group recommends that the foam should either be replaced wherever it has been used in the Browns Ferry or other nuclear plants, or that special safety procedures should be set up if this is not possible. More generally, the group says that, better all-round fire prevention measures are needed in nuclear plants.

Fire fighting procedures at the Browns Ferry also come in for criticism. The report points out, in particular, that the part of the fire in the reactor building "was fought unsuccessfully for several hours with portable CO_2 and dry chemical extinguishers; however, once water was used, it was put out in a few minutes". The old maxim about not using water on electrical fires has to go quickly by the board in such cases if other means clearly fail, the report concludes after considering the views of fire experts.

The fact that several of the plant's control systems were damaged in the fire, including redundant systems that should have prevented complete loss of any function, also led the group to criticise other features of the Browns Ferry design (which, to be fair, was carried out in the mid-1960s). For example, wiring connected to some redundant devices was all located close together, with one set simply placed in a conduit. In spite of this, the fire damaged both sets of wires in several instances.

The group recommends that the NRC should sharpen up many of its procedures for developing standards, making inspections and so on, but points out that the very small probability of accidental release of radioactivity from such a fire is "about 20%" of that calculated from all other possible causes. Thus "urgent" action to reduce fire risks is not required, it says, and in particular there is no question of shutting down other plants while minor modification work is carried out.

The Browns Ferry reactors are expected to start generating electricity again in April.

● A paper released by the Washington-based Worldwatch Institute (*Abortion Liberalisation*: *A Worldwide Trend*) reports that 64% of the world's people live in countries where abortion is liberally available. Five years ago, in January 1971, the proportion was 38%, and writers Lestor R. Brown and Kathleen Newland say that "few social changes have ever swept the world so rapidly".

The worldwide movement, they argue, "reflects an increasing willingness by national legislatures to face the reality of abortion as a public health issue"—but the public health benefits of safe abortion depended on its availability in fact as well as in legal theory, which made cost and distribution important. Women would also continue to seek abortion, even when illicit and dangerous, while there was a lack of failure-proof and problem-free contraceptives. ●

FRANCE

The agreement reached at the recent Nice summit between the French and West German Technology ministers on cooperation in nuclear reactor technology, while vague, represents a pooling of risks, regarding both fast breeders and high temperature reactors, that both countries hope will work to their mutual advantage.

The indications are that Germany will benefit in the sphere of fast breeders. Her operation, conducted along with the Benelux countries, is racked by rising costs and due to bear fruit only in 1981, while France's 250 MW Phenix prototype sodium-cooled reactor has been working successfully for a year. In the field of high temperature reactors, on the other hand, Germany's project, although also behind schedule and increasingly costly, is one of the few of its type, and the Schmidt government appears prepared to take it further.

The money involved, of course, is enormous, and it is not clear whether financial backing will be divided equally between the two areas. It is expected that the two countries will contribute equally, but the agreement declares only that they should spend the amounts on the two areas taken together.

The conclusion came just a few weeks after the completion of a deal between France and the Westinghouse Electric Corporation which also reflected a trend to "Europeanise" the nuclear industry this side of the Atlantic. Westinghouse is to sell two-thirds of its 45% holding in Framatome, the French licensee of its pressurised light-water reactor, to the French Atomic Energy Commission (CEA) for $25 millions.

Westinghouse will transfer its remaining 15% Framatome interest to the Creusot-Loire Company, the heavy equipment suppliers with a 51% interest in Framatome, in 1982, when the licenses expire. Until then, Westinghouse will be able to buy up to 1,200 tonnes of uranium from the CEA. Westinghouse will also continue to receive royalties for Framatome's use of Westinghouse technology in fulfilling orders and options, which now extend to over 40 plants. Westinghouse will take part in a joint research and development programme as well.

This "partnership" through Framatome between the CEA and Creusot-Loire in respect of pressurised-water reactors could conceivably expand into the field of fast breeders with the Franco-German agreement. Cooperation between the two countries on fast breeders, it is understood, will take place through a joint company. On the German side will be Kraftwerke Union or a subsidiary; on the French side will be the CEA, or possibly Novatome, which is owned by the CEA and Framatome together with the Compagnie Generale d'Electricite (CGE). The CGE was involved in the ill-fated boiling-water reactors about which the government last year changed its mind; but, more importantly, it has a stake in a company working on the French fast breeder, GAAA, in which Creusot-Loire has been interested in order to widen its arena of nuclear activity. The size of the respective stakes appear to make it possible for CGE resistance to Creusot-Loire to be circumvented through Novatome.

Photo: Cam & Pen Int

● Another important deal which, like that involving Westinghouse, took months to finalise, was completed just before the New Year. An agreement between the American computer firm Honeywell Information Systems and the French government merges Honeywell's French subsidiary with the general-purpose business of the national computer company, CII, to form CII-HB, 20% owned by the government and, altogether, 53% owned by French interests.

The government is expected to give about $280 millions of subsidies over four years, and a government-sponsored finance company will purchase computers sold to the government and nationalised industries. Furthermore, the addition of the 1,300 employees of the CII Research and Development Engineering Department will enhance the research resources of the new company. The whole deal represents the third attempt in eight years to establish a French computer industry that can compete with IBM in the country. But it also represents a setback for attempts to forge an overall European solution.

● The 15-year period after 1955, an era of strong growth in research which coincided with considerable expansion of the French university system, has been followed by a recession which has left university research facing very serious problems.

Now, following the creation of an autonomous National Secretariat for Universities in 1974, ostensibly to oversee both CNRS and university-financed research, the government has belatedly laid some foundations for organising university research policy. A central role has been accorded to the Secretary of State for the Universities (Mme A. Saunier Seité, who replaced M J. P. Soisson in January's ministerial reshuffle) in overseeing co-ordination of university research—an objective that has been sought for a decade. Groups have been formed within the National Secretariat for this purpose, but the overall effort is being directed towards refining budgetary procedures that permit healthy growth as well as adjudicating between university research programmes.

The driving force behind all basic research is the National Scientific and Research Centre (CNRS), with a budget (in 1976) of about 21,000 million francs. It directly finances the major part of research in universities, with which its links are close; the universities' own budget for research last year amounted to an estimated 1,400 million francs, a figure which includes part of the salaries of professors and assistants.

University research which is independent of the CNRS faces typical problems. Laboratories unaided by the CNRS have seen the buying power of their finances eroded since 1969. Moreover, insufficient coordination at the national level has helped to diminish the credibility of an often vague (even non-existent) university research policy. Research teams in the smaller and medium-sized provincial universities have also faced difficulties attaining the necessary size, although all the universities are now feeling the crisis, including such large Parisian universities as the Pierre and Marie Curie University.

● At the end of January, just a fortnight after the court established by France and Britain began considering demarcation between their off-shore oil prospecting limits, on which a decision is expected in the summer, reports emerged of hydrocarbon finds at a depth of 5,840 feet off the Brittany coast, though not in a disputed area. Results of commercial tests are awaited, but drilling will continue to 9,850 feet.

IN BRIEF

Geothermal energy

Geothermal energy in the United Kingdom is not likely ever to be a major resource. This was the unsurprising conclusion to be drawn from evidence recently given to the Energy Sub-Committee of the Commons Select Committee on Science and Technology by Dr Bill Bullerwell, Deputy Director of the Institute of Geological Sciences. Even the few prospects worth pursuing, particularly in Cornwall and the West Hampshire Basin, did not warrant more than a proposed £800,000 research programme over the next three years. In the longer run Dr Bullerwell thought there were possibilities in disused North Sea wells, where temperatures up to 160 °C have been recorded; water might be circulated and the heat converted to hydrogen storage.

UK energy policy

The UK Department of Energy is aiming for a bigger Government say in shaping the country's future energy policy. The changes it proposes come in a 20-page report circulating among Government and trades union officials which was prepared by the political advisers to the Energy Secretary, Mr Anthony Wedgwood Benn. The report follows his call last week for a new approach to long-term planning, and suggests the introduction of new administrative set-ups within the nationalised fuel industries.

Telecommunications research

The UK telecommunications industry entered the telephone centenary week with a sharp reminder of its "competitive weaknesses". The Labour Research Department, an independent, union-backed organisation, has suggested that the fault lies in the comparatively low levels of telecommunications research spending in Britain, and sees one possible remedy in the extension of state control to the private supply industries.

Saving the wetlands

KENNETH MELLANBY

MAN has modified the natural landscape in all parts of the world, to make it more productive and to support a larger human population. He has felled the forests which originally covered most temperate areas, and he has drained marshes and fens to produce fertile farmland. In the past these activities were almost universally applauded. In the seventeenth century the Dutch engineer Vermuyden introduced scientific land drainage to East Anglia; he was hailed as a benefactor for turning the almost unproductive wasteland into some of the best arable in England. Others who extended his work, including the drainage of Whittlesea Mere, the largest lake in Southern England, were seldom criticised for their actions. Almost everyone thought that agricultural improvement should be encouraged.

The only objectors to drainage were the few "fen tigers", poor peasants who made their living catching eels and wildfowl, and cutting reed for thatch. Today, when most of Europe's marshes have been drained, and development is destroying (or improving) wetlands throughout the world, the ecological importance of such areas is being increasingly realised. We have the international "Convention on Wetlands of International Importance", and this year, 1976, the Council of Europe is sponsoring a European Wetlands Campaign. The intention is to try to preserve as many as possible of the remaining and unpolluted rivers, estuaries, lakes, ponds, marshes, bogs and fens as habitats for wildlife, particularly for waterfowl.

Agriculture is perhaps no longer the main cause of wetland destruction, though the unwise use of pesticides on crops near to rivers and marshes has taken its toll of fish and fish-eating birds. Industrial pollution has also had its effect on all forms of wildlife. Marshes near to human settlements, particularly in the tropics, are blamed for breeding mosquitoes which transmit malaria and other diseases, and may be saturated with DDT and other chemicals. Uncontrolled shooting has affected bird populations, and obtrusive sports like water skiing and speedboat racing have turned peaceful lakes into noisy infernos.

It would be absurd to pretend that the majority of the population of any developed country cared greatly for wetlands and their flora and fauna, and public concern in developing countries must be minimal. So the conservationists must be congratulated in obtaining such widespread international support, from scientists and politicians, for their preservation. However, though many of our governments pay lip service to this type of conservation, their actions have been incredibly dilatory and have not always accorded with their public protestations.

Although there have been international discussions on wetland and waterfowl conservation for decades, it was only in February 1971 that the text for a convention was formulated at Ramsar in Iran. It was then agreed that when seven different countries had ratified this convention, it would come into force. Bureaucracy has delayed this ratification for five years, during which period many valuable wetlands which might have been saved have been lost.

This delay has been particularly discouraging for the British ornithologists and conservationists who played a leading part in the whole campaign. They hoped that Britain would set an example by rapid action, so leading the world officially as well as scientifically. This was not to be. The convention was first ratified by Australia, then by Iran, Finland, Norway, Sweden and South Africa. This was the state of play in March 1975. One more signature was needed —surely this would be that of Britain. At a meeting in Germany months earlier the Foreign Office had assured our delegation that ratification was "imminent". However, Greece acceded in August 1975, bringing the convention into force. Bulgaria signed next, Britain came in ninth. Now Britain is used to seeing its representatives coming in far down the line at the Olympic Games, so perhaps we should not have been surprised to find our diplomats are as slow as our athletes.

However, let us be thankful for small mercies, in that, at long last, the Foreign Office has given its support to the efforts of our scientists. No doubt the official responsible will be suitably rewarded. As he has been concerned with fens and bogs, the most appropriate honour would be the prestigious Royal Victorian Chain; this rarer decoration would clearly be more suitable than the commoner Order of the Bath.

correspondence

Financial discrimination

SIR,—As is shown by a survey I made recently, reported on by May (February 12, page 446), many journals discriminate in various ways against the more or less impoverished. I publish a journal myself and have grounds for believing that such discrimination is unnecessary. It preserves showiness (at the expense of economies which leave the scientific content intact) and profit. Editors of journals are usually not impoverished, and the present system does have real advantages to those with money.

As with other socially irresponsible powers, a remedy requires joint action. I am therefore pursuing the following actions, and call on others to do likewise:

- To submit no paper to an irresponsible journal except when there is no reasonable alternative.
- To avoid citing papers published after the end of 1976 in irresponsible journals, when alternative citations are available.
- To refrain from refereeing papers for irresponsible journals.

Some journals are more irresponsible than others, and one can be more stringent with the worse ones.

Yours faithfully,
LEIGH VAN VALEN

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Sex laws and inheritance

SIR,—The sexual discrimination laws now in force in the UK should not blind us to the existence of one social inequality which has a sound physiological basis I mean the nature of inheritance.

In general, any one of our chromosomes (or any part of one) may have come from any one of our grandparents: no fragment of genetic material can be identified with a particular grandparent, with the unique exception of the Y chromosome in men. A man's Y chromosome is certainly derived from his father and from his paternal grandfather and so on. No other chromosome has this singular property.

Consider in particular a woman's X chromosomes. They assuredly were derived one from her mother and one from her father. But the maternal one —is it in turn from her maternal grandmother or grandfather? It could be from either. Her paternal X chromosome is certainly derived from her paternal grandmother but its pedigree similarly stops short.

Quite generally, any of our forebears, from our grandparents back, may be completely unrepresented in our chromosomes (although crossing-over reduces the probability of any such omission).

Therefore, if a woman and her husband intend only their (genetic) descendants to receive their name, title or family silver, they had better ensure that it goes only to their sons and their sons' male descendants.

Yours faithfully,
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Journal guidelines

SIR,—As Professor Ziman remarks (January 29, page 264), evidence for or against the anonymity of referees is difficult to present. However, my claim that six years editorial experience confirmed the validity of the initial policy statement in the *Journal of Aerosol Science* can be enlarged on There were three reasons for identifying referees:

- The authoritarian age is over. Professor Ziman is in error in identifying this as a populist rejection of constraints imposed by the community. It refers, of course, to constraints imposed by a favoured section of the community.
- The standard of critique will be raised. By eliciting specific criticisms in place of diffuse; by 'curbing the vanity and pride of those who claim authority'; by disclosing a possibly partisan opinion in a debated field; by revealing a possible error in referee selection
- Encouragement and benefit will result to authors. The young scientist may be put in touch with a well-known figure; an argument between equals becomes definable; a senior worker should know the source of critical comment, especially when it comes from a younger person.

The opposing view of Professor Ziman rests largely upon emotional factors. I do not believe that they are exacerbated by disclosing a personal target—rather the reverse. One would expect a referee to excuse himself if asked to deal with the work of a friend or rival were the editor ineptly to place him in an embarrassing situation.

Yours faithfully,
C. N. DAVIES

University of Essex,
Colchester, UK

Fruit flies as security officers

SIR,—Trained dogs have an established role as aids to Customs Inspectors and Airport Security Guards because of their ability to "smell out" concealed drugs or explosives or other forms of contraband. What seems to have been overlooked is the possibility of using insects for the same purpose.

The simpler insects have genetically fixed responses to specific odours, usually resulting in a conspicuous clustering in the vicinity of the odour source which may be of an extremely low absolute intensity. Kikuchi (*Jap. J. Genet*, **48**(2), 105; 1973 and *Nature*, **243**, 36; 1973) isolated a mutant strain of *Drosophila melanogaster* that was strongly attracted to eighteen chemicals which were repellent to the parent strain. It seems likely, therefore, that a systematic search for other strains selectively responsive to various narcotic or explosive emanations can uncover specific "olfactory mutants" of a practically useful sort.

While there would be obvious objections to using such species as cockroaches, bees or houseflies, the relatively inconspicuous but not unfamiliar fruit flies would probably be an acceptable alternative to some of the existing security checks

Yours faithfully,
R. H. WRIGHT

6822 Blenheim Street,
Vancouver V6N 1R7, Canada

The JPU in modern science

SIR,—In view of the current interest in citation-counting and other attempts to quantify scientific achievement, I wish to draw attention to a simple concept which may grow in importance in the future This is the JPU, or "Just Publishable Unit", which may be defined as the smallest amount of information which is normally accepted for publication as a separate item in a scientific journal. An example is the present communication.

Yours faithfully,
D FRASER

Ministry of Natural Resources,
Maple, Ontario, Canada

news and views

Molecular analysis of the oncogene

from Robin Weiss

GENETIC evidence that avian sarcoma viruses carry a specific gene for neoplastic transformation of the host cell has steadily accrued over the past five years. Martin (*Nature*, **227**, 1021; 1970) first isolated mutants of Rous sarcoma virus (RSV) which were temperature-sensitive for maintaining the transformed state of the cell, but which were not temperature-sensitive for viral replication. Then Vogt (*Virology*, **46**, 939; 1971) and Martin and Duesberg (*Virology*, **47**, 494; 1972) observed the spontaneous segregation of transformation-defective variants of RSV that were subsequently shown to be deletion mutants (Lai *et. al.*, *Proc natn. Acad. Sci. U.S.A.*, **70**, 2266; 1973) lacking 10–20% of the viral genome. Recent genetic analysis indicates that all temperature-sensitive transformation mutants represent a single gene (Wyke, Bell and Beamand, *Cold Spring Harbor Symp. quant. Biol*, **39**, 897; 1975) which map in the region deleted from the transformation-defective mutants (Martin and Bernstein, personal communication). Thus avian sarcoma viruses appear to carry a gene (called *sarc*) which is quite superfluous for viral replication, but which is essential for the maintenance of the malignant state of fibroblasts.

Stehelin, Guntaka, Varmus and Bishop (*J. molec. Biol*, **101**, 349; 1976) have prepared a specific cDNA$_{\text{sarc}}$ probe complementary to the sarc gene of the virus. This was accomplished by transcribing complementary DNA of non-defective RSV by its endogenous RNA-directed DNA polymerase activity, followed by molecular hybridisation to the RNA of a transformation-defective deletion mutant. The non-hybridised cDNA, which can be separated on a hydroxyapatite column, represents a uniform transcript of the deleted sequences. Nucleotide sequences homologous to avian cDNA$_{\text{sarc}}$ seem to be present in all avian sarcoma viruses, but are absent from avian leukosis viruses, and from sarcoma and leukaemia viruses of mammals. On page 170 of this issue of *Nature*, Stehelin, Varmus, Bishop and Vogt demonstrate that cDNA$_{\text{sarc}}$ hybridises with DNA from normal chicken cells

Previous studies by Neiman, *et al.* (*J Virol*, **13**, 837; 1974) indicated that the hybridisation of sarcoma virus RNA to normal chicken DNA was entirely blocked in the presence of excess RNA from transformation-defective viruses, suggesting that sarcoma-specific sequences were not present in the normal chicken genome. The discrepancy between their results and those of Stehelin *et al.* remain to be explained. Thermal denaturation experiments by Stehelin *et al*, however, show a 4 °C depression in melting temperature (Tm) of cDNA$_{\text{sarc}}$ duplexes with normal chicken DNA by comparison with the Tm of duplexes with the provirus of RSV. This indicates substantial mismatching between the viral sarc sequences and those endogenous in the chicken cell. Under Neiman's more stringent conditions of hybridisation, such partially homologous sequences might not be detected.

Hybridisation of cDNA$_{\text{sarc}}$ to DNA of other avian species reveals a widespread occurrence of sarc-like sequences in normal DNA, including that of a primitive ratite bird, the emu. The melting temperatures of the duplexes indicate a divergence of sarc sequences roughly in parallel with species divergence, in contrast to a much more rapid divergence of endogenous leukosis virus gene sequences. Stehelin *et al.* anticipate that the cellular DNA related to cDNA$_{\text{sarc}}$ serves some function which accounts for its conservation during avian speciation. The sequences are part of the unique fraction of cellular DNA and could represent either structural or regulatory genes. The sarc gene of the virus may have been acquired from a cellular genome, possibly (in view of the melting curves) from a species other than the chicken

It is not yet clear whether the sarc-like sequences in normal avian cells are specifically expressed in normal embryogenesis or following non-viral carcinogenesis, as suggested by Huebner and Todaro when they enunciated the viral oncogene concepts (*Proc natn. Acad. Sci. U.S A.*, **64**, 1087, 1969) Bishop and Varmus' laboratory is currently studying this question

The widespread occurrence of sequences related to cDNA$_{\text{sarc}}$ in avian species will doubtless stimulate more intensive studies of sarc sequences in mammalian sarcoma viruses. Scolnick, Parks and colleagues (*J. Virol.*, **12**, 458; 1973; **13**, 1211; 1974) have detected sarcoma-specific sequences in different strains of murine sarcoma virus (MSV). In the Kirsten and Harvey strains, the sarc sequences, plus other viral sequences, seem to be derived from a rat host, whereas Moloney strain MSV is related to the mouse only. It will be interesting to find out whether the 'sarc' sequences of feline and simian sarcoma viruses are related to those of MSV, and, by analogy with the wide phylogenetic representation of sarc sequences among avian species, whether any of these sarcoma viruses will be useful in probing sarc-specific expression in human tumours.

There is no genetic evidence so far of specific oncogenes in leukaemia viruses of any kind. Viruses that are typically leukaemogenic *in vivo* but can also transform fibroblasts *in vitro*, such as murine Abelson virus and avian MC29 virus, do not seem to carry sarc sequences (Scolnick *et al.*, *Proc. natn. Acad. Sci. U S.A.*, **72**, 4650; 1975; Graf and Stehelin, personal communication), and neither are sarc sequences detectable in the cellular RNA of Abelson-transformed fibroblasts. Thus these viruses may transform cells by a different mechanism or gene product.

If the viral sarc sequences do code for a specific protein, it may be possible to identify it by comparing *in vitro* translation products of sarcoma viruses with those of their transformation-defective deletion mutants. Such attempts by several laboratories have not, however, yielded an identifiable protein so far. Of course, the isolation of an 'oncoprotein' coded by the 1,600 or so nucleotides of the 'sarc' gene is a major goal of oncornavirologists today How such a protein might interact with the host cell is an open question. Some virologists expect it to be a nuclear protein which would effect regulation of DNA synthesis, whereas others feel it may act elsewhere, by insertion in the cell membrane, for example. In this case the transformation-specific cell surface antigen of virally transformed avian cells is already a candidate Since a nuclear antigen corresponding to papovavirus T antigen or EBNA of cells transformed by Epstein-Barr virus has not been identified in cells transformed by sarcoma viruses, I currently favour a non-nuclear role.

Maternal effects on development

from Michael H. C. Snow

During the past 50 years the attention of embryologists has been much taken up with induction and activation. For them these terms are descriptive of the intercellular and intracellular processes whereby, presumably as the result of information received (or lost), a tissue or cell is made to modify its course of development. To the bacterial geneticist, however, induction and activation imply the mechanisms involved in the control of gene expression. Because cell differentiation depends on qualitative changes in gene activity, the terms should eventually come to mean the same to both groups. But at present the bacterial geneticist can refer to precise interactions between inducing or activating molecules and the genes themselves, while this is not possible for the embryologist dealing with higher organisms

The current state of knowledge of these processes in embryonic development is brought into sharp focus by two recent papers. Toivonen *et al.* (*Differentiation,* **5**, 49; 1976) consider inductive interactions between tissues. Primary induction whereby neural tube formation by the ectoderm is triggered by the underlying mesoderm was discovered, in Amphibia, by Spemann and his colleagues in the 1920s. They later presented evidence that an unidentified chemical substance(s) produced by the mesoderm was the effective agent (Bautzmann *et al., Naturwissenschaften,* **20**, 971; 1932). Since then however, the existence of diffusible inductive agents has been the subject of much controversy, partly because of the difficulty experienced in repeating some of the original experiments, and more recently because Saxén and coworkers demonstrated the necessity for cell contact between tissues in the induction of kidney tubule formation (Lehtonen *et al., J. Embryol. exp. Morphol.,* **33**, 187; 1975). Nevertheless, it is now clear that cell contact is not required for the initiation of primary induction and that some diffusible macromolecular factor is involved. The exact nature of the inducer and its mode of action is still obscure.

Most if not all embryos depend for their early development on material deposited in the oocyte cytoplasm, which is subsequently used during cleavage stages. In some cases maternally derived cytoplasmic factors are required for the normal development of much later stages. Deficiences in the oocyte cytoplasm (for example, those resulting from mutation in the maternal genome) can therefore profoundly affect the development of the embryo. Several such maternal effect mutations are known in various organisms, such as the grandchildless mutant in *Drosophila pseudoobscura* (Spurway, *J. Genet.,* **49**, 126; 1948), shell coiling in the snail *Limnaea* (Conklin, *Anat. Anz ,* **23**, 577; 1903) the *O* mutant in the axolotl (Briggs and Cassens, *Proc. natn Acad Sci. U.S.A.,* **55**, 1103; 1966) and a recent example has been described in the DDK strain of mouse (Wakasugi, *J. Reprod. Fert.,* **33**, 283; 1973).

The interest in maternal effects stems from the observation that abnormal development occurs although the genetic constitution of the individual is normal. The error apparently results from a failure to activate certain genes during embryogenesis.

A particularly exciting example of a maternal effect gene, the *O* gene in the axolotl is the subject of Brothers' report in this issue of *Nature* (page 112). The crucial observations on the *O* gene are as follows. (1) Development is arrested in embryos derived from homozygous O females at the onset of gastrulation At this time much of the embryonic genome becomes active, in particular the genes coding for ribosomal RNA. (2) The product of the normal allele (O^+ factor) can be removed from the oocyte and on iniection into mutant eggs can correct the arrest at gastrulation. (3) The O^+ factor is a protein and curiously is most abundant in the oocyte nucleus. What Brothers has shown, by a series of very elegant nuclear transplant experiments, is that the time of action of the O^+ factor is restricted to a very short period in development of a few hours in the mid-blastula stage If the O^+ factor can be isolated and purified then the way is open to analyse the manner in which at least one inductive agent operates.

In this context it is worth noting some of the other changes found in embryos at the end of cleavage. For instance, a change has been reported in the nature of histone proteins in *Drosophila melanogaster* (Das *et al., J. Cell Biol.,* **23**, 423; 1964), in the snail, *Helix aspersa* (Bloch and Hew, *J. biophys. biochem. Cytol ,* **8**, 69; 1960) and in the mouse (Alfert, *Expl Cell Res., Suppl.* **6**, 227; 1958). Histones are known to be potent but nonspecific repressors of transcription. Non-histone proteins on the other hand, have been postulated as being involved in derepression (activation) (Wang and Kostraba in *The Role of RNA in Reproduction and Development,* (edit. by Niu and Segal) 324; North Holland, Amsterdam, 1973).

The onset of gastrulation is generally accompanied by a significant change in cell proliferation rate, and in the mouse this is coincidental with a change in chromosome replication behaviour (Takagi, *Expl Cell Res.,* **86**, 127; 1974, Snow, *Ciba Symp ,* **40**, in the press). Callan has shown that in Amphibia at least, large changes in the duration of the DNA synthesis phase of the cell cycle are likely to be associated with changes in replicon length rather than changes in the linear rate of DNA replication (Callan, *Proc. R. Soc., Lond.,* **B181**, 19; 1972, and in *Molecular Cytogenetics* (edit. by Hamkalo Papaconstantinou, 31, Plenum, 1973). DNA involved in replication is not available for transcription and the restriction in the number of replicating forks in the DNA may be necessary to permit transcription of large amounts of RNA. The O^+ factor could operate in either of these areas, or alternatively could be very much more specific and result perhaps in the activation of a specific gene. After 50 years of probing, a breakthrough on any front would be welcome. ☐

Role of ribosomal RNA

from Richard Brimacombe

The flurry of interest in bacterial ribosomal proteins over the past few years has tended rather to obscure the fact that about 60% of the bacterial ribosome consists of RNA, and many workers have tried to relegate the rRNA to a mere structural component. This attitude has arisen partly as a consequence of the dogma that proteins and not nucleic acids are the active agents in biological systems, but is also a result of the fact that, in contrast to the studies which can be made with individual ribosomal proteins, it is very difficult to devise functionally oriented experiments involving the large rRNA molecules. The idea that rRNA does indeed have an important functional role is of course not new; it was suggested some years ago by Crick and

On the lagoon

from Gillian Boucher

A NEW study of the behaviour of the tide within the lagoon of Venice, in itself not a revolutionary idea, promises to have far-reaching practical results in the Italian government's choice of the most suitable barrier to prevent flooding in Venice.

A. Goldmann, R. Rabagliati and P. Sguazzero of the IBM Scientific Centre in Venice in an article in the *Rivista Italiana di Geofisica* (**2**, 119; 1975) have computed the relation between the level of water outside the lagoon and the level at 14 points within the 50 km^2 lagoon. The data were provided by hourly readings of water level at the 15 stations during 1972 and 1973. Using spectral analysis, the authors first investigated the transfer of energy from the open sea to every lagoon station at each of a large number of frequency bands. This showed that only in three frequency bands—the long-term, the diurnal and the semi-diurnal—was the transfer of energy significant. The authors then calculated the gain or loss in the amplitude of the diurnal and semi-diurnal waves—those in which the astronomical components are predominant—and the time it took waves of these two frequencies to reach each station (the phase).

In 1971 the lagoon was sounded in detail: the soundings revealed that 75% of the lagoon is shallow water or marsh less than 2 m deep, intersected by the deep channels which make up the other 25%. When this information is combined with the tide data an interesting picture begins to emerge: on deep channels the semi-diurnal waves, although not the diurnal waves, actually increase in amplitude after entering the lagoon (the gain at the Grand Canal station is 6%); in shallow water on the other hand the waves lose amplitude, the semi-diurnal waves losing more amplitude than the diurnal ones. Propagation of waves is slower in shallow water and the waves of the diurnal tide are propagated more slowly than those of the semi-diurnal.

The detailed results, with the advantages of a long period of data collection, the almost contemporary sounding data, and sophisticated statistical techniques, provide a clearer picture of the lagoon than has ever been obtained before. One important use for them will be to show how the lagoon is changing by comparison with past and future results. But the results have already been used in another of the IBM projects, very recently completed· a mathematical model of the entire lagoon. The model, calibrated by the new tide results and other information, can reproduce the behaviour of water at any point and the effects of man-made changes in the lagoon. One of the first problems the model will be called upon to solve is the effect upon the lagoon of the various types of barrier which could be erected at its three entrances.

One of the scourges of Venice is the tide which, though small by Atlantic standards, frequently causes flooding because the city is so low-lying. To save the city from the salt-water inundations the Italian government has invited tenders for the construction of a sluice at the entrances to the lagoon; companies interested in tendering must do so by June 30 this year. Of the two basic types of barrier, the moveable and the fixed, the moveable (for example Pirelli's project for an inflatable barrier) are clearly superior but very much more expensive. A fixed barrier completely shutting off the lagoon is out of the question—Venice relies on the tide to wash away industrial and domestic pollution, and to prevent circulation of water would be devastating to both humans and buildings. So the choice is between a moveable barrier and some kind of partial fixed barrier which will lessen the quantity of water entering sufficiently to prevent flooding. Inevitably a fixed barrier will also reduce circulation. The IBM model will enable companies and the government to decide whether a satisfactory compromise can be obtained between the needs to damp the tides and to control pollution.

Orgel (*J molec. Biol.*, **38**, 367 and 380; 1968) that the primitive ribosome consisted primarily of RNA, with the proteins being added later to fulfil more sophisticated functions. More recently, the functional importance of RNA in the "modern" ribosome has been preached by a number of authors, most notably by Kurland (*Ribosomes*, 309, Cold Spring Harbor Monograph, 1974), and the purpose of this article is to review very briefly the present status of rRNA, with regard to both structure and function.

Little progress can be made nowadays in any nucleic acid problem without previous knowledge of the base sequences involved. In the *E. coli* ribosome, the sequence of the 5S RNA component has been known for many years (Brownlee *et al.*, *J. molec. Biol.*, **34**, 379; 1968), and the primary structure of the 16S RNA is now almost fully established (Ehresmann *et al.*, *Nucleic Acids Res.*, **2**, 265; 1975), although there is still a measure of disagreement as to the detailed sequences of some of the oligonucleotide degradation products (Magrum *et al.*, *Nature*, **257**, 423; 1975). Progress has also been made in the sequencing of the 23S RNA (Branlant *et al*, *Biochimie*, **57**, 175; 1975). The primary sequences themselves give a strong hint that the RNA is more than just a structural skeleton within the ribosome; the 5S sequence has been strongly conserved throughout evolution (reviewed by Monier, *Ribosomes*, 141, Cold Spring Harbor Monograph, 1974), and comparison of the oligonucleotide catalogues from 16S RNA molecules from different bacteria shows a remarkable degree of homogeneity (Woese *et al.*, *Nature*, **254**, 83; 1975), again indicating that substantial regions of the sequence have been conserved. The obvious inference is that particular oligonucleotide sequences are required to fulfil specific ribosomal functions, and this has tempted several authors to put forward hypotheses involving complementary base-pairing between regions of rRNA and other nucleic acid components of the protein synthesising system (for example, van Knippenberg, *Nucleic Acids Res.*, **2**, 79; 1975). Although these ideas are attractive, it must be borne in mind that in molecules the size of rRNA, any given nucleotide sequence of up to six bases would be expected to appear on a statistical basis, and solid experimental evidence to support this type of hypothesis is unfortunately very difficult to obtain.

Evidence has however been forthcoming in two cases. The first of these concerns the proposal made by Shine and Dalgarno (*Proc natn. Acad Sci. U.S A.*, **71**, 1342; 1974) that the 3′-terminal sequence of 16S RNA is involved in mRNA recognition. Here, Steitz and Jakes (*Proc. natn Acad. Sci. U.S.A.*, **72**, 4734; 1975) have been able to isolate a dissociable oligonucleotide complex consisting of the 3′-terminus of the 16S RNA and an RNA fragment from a protein initiator region of R17 phage RNA (see also News and Views, **260**, 12; 1976). The two fragments contained a seven-base complementary sequence The second case concerns the 5S RNA, where several lines of evidence suggest that binding of tRNA to the 50S ribosome involves a complementary base-pairing between the TψCG loop of the latter with 5S RNA (Erdmann *et al.*, *Biochem, biophys. Res. Commun*, **54**, 942; 1973). Here there is some conflicting evidence, as the relevant G-residues on the 5S RNA are not exposed (within the ribosome) to attack by kethoxal, a reagent which specifically attacks non-paired guanine bases (Noller and Herr, *J. molec. Biol.*, **90**, 181; 1974). This difficulty can of course be overcome by postulating appropriate conformational changes (Schwarz *et al.*, *Biochem. biophys, Res. Commun.*, **56**, 807; 1974).

Kethoxal has been successfully used as a probe for the accessibility of G-residues on the surface of the 30S ribosomal subunit (Noller, *Biochemistry*, **13**, 4694; 1974), and these studies have also provided information regarding the vexed question of the secondary structure of the rRNA. There is still no proven secondary structure for the 5S RNA, and, while a secondary structure has been proposed for *E. coli* 16S RNA (Ehresmann *et al.*, *Nucleic Acids Res.*, **2**, 265; 1975), there is at present little evidence to support it. Indeed the kethoxal data are to a considerable extent at variance with the proposed structure, and a further complication has recently arisen with the finding that specific regions of the 16S RNA are involved in stable long-range interactions with other parts of the molecule (Mackie and Zimmerman, *J. biol. Chem* , **250**, 4100; 1975). This suggests that secondary structure model-building based on a simple linear array of hairpin loops may be *a priori* invalid.

Many questions concerning both secondary structure and function of the rRNA will perhaps become clearer with a better understanding of the interactions between RNA and protein within the ribosome. This is one of the most important problems of ribosomology, but again it has not proved an easy one to approach. Most of the available data has been obtained by Garrett and Zimmermann and their co-workers from synthetic complexes between single ribosomal proteins and RNA (reviewed by Zimmermann, *Ribosomes*, 225, Cold Spring Harbor Monograph, 1974). These complexes are subjected to a mild nuclease digestion, following which the RNA region "protected" by the protein can be identified. The approach is of course limited to a study of those proteins which can individually form specific complexes with RNA, the "RNA binding" proteins, but it has nevertheless provided a great deal of information in a number of cases. The size and complexity of the RNA regions involved varies considerably; at one end of the scale are proteins such as S8 and S15 which are found in association with short regions of 16S RNA of the order of 50 nucleotides long. Similar short regions of 5S RNA have been found corresponding to proteins L18 and L25. At the other end of the scale are proteins S4 and L24, both of which interact with very large sections of 16S or 23S RNA approximately 400 nucleotides in length. How far these "binding sites", in particular the very large ones, reflect the situation within the intact ribosome remains to be seen

A hundred years ago

WE cannot see the force of some of the arguments with which Captain Moresby supports his plea for annexation [of New Guinea]. His strong attachment to the natives and his desire for their welfare we think mislead him as to how this is to be accomplished. If New Guinea is to be colonised by white men, all previous experience teaches us that the natives will inevitably suffer, will be demoralised, and ultimately extinguished. It is inexpressibly sad to think of such a fate overtaking these gentle and altogether superior natives of New Guinea; but how can it be helped, unless it is resolved to put a stop to the increase in the white portion of the world's population.

from *Nature*, **13**, March 9, 365; 1876.

For the examination of RNA–protein interactions within the ribosome itself, especially those involving proteins which are unable to bind individually to RNA, another approach is necessary. One method has been to isolate fragments from the complete ribosome following nuclease digestion, and to analyse those RNA sequences which interact with particular groups of proteins in the fragments (for example, Yuki and Brimacombe, *Eur. J. Biochem.*, **56**, 23; 1975); further development of this approach has however been severely limited by the ease with which the specificity of the RNA–protein interaction is lost. A more promising approach, now being undertaken by a number of laboratories, involves the development of suitable RNA–protein cross-linking techniques. One example has been the cross-linking of proteins to the 3′-terminus of the 16S RNA, by way of the periodate oxidation of the 3′-terminal ribose. The proteins concerned are S1 and S21 (Czernilofsky *et al.*, *FEBS Lett.*, **58**, 281; 1975), which is interesting in view of the foregoing discussion of initiation, since both these proteins are involved in mRNA binding. S1 has also been shown to bind to an RNA fragment from the 3′-terminus of the 16S RNA (Dahlberg and Dahlberg, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2940; 1975).

Cross-linking of protein to RNA within the bulk of the rRNA requires more general methods, but here also a measure of success has been recorded, such as the identification of some peptides of protein S4 which are in contact with the 16S RNA (Ehresmann *et al.*, *FEBS Lett.*, **58**, 106; 1975), or the identification of a region of 16S RNA which is in contact with protein S7 (Rinke *et al.*, *Eur. J. Biochem.*, in the press), in both cases after cross-linking induced by ultraviolet irradiation. These techniques clearly need a lot of refinement, but an extension of this type of study, coupled with the development of more sophisticated cross-linking reagents, seems at the moment to hold the most promise for the immediate future. The recent application of various physicochemical techniques to the protein topography problem is very rapidly giving us a firmer knowledge of the spatial distribution of the ribosomal proteins; it will not be too long before we have at least a first approximation as to how the rRNA is threaded through this protein matrix. This would be invaluable in studies of both structure and function.

Models from maps

from S. D. Dover

ANALYSIS of the electron density maps which are the results of an X-ray crystallographic investigation is the last stage in protein structure determination.

In the initial development of the subject maps were analysed by placing markers in regions of density corresponding to an identifiable feature, measuring the marker co-ordinates, and adding to or adjusting a wire model of the structure accordingly. The interpretation of a new part of the map frequently relied on the existing portion of the model This feedback was facilitated by the Richard's box (*J. molec. Biol.*, **37**, 225; 1968) a construction which enabled an image of the model in a half-silvered mirror to be superimposed on a stack of perspex sheets on which were drawn the electron density contours. Alterations or additions to the model were then seen directly in the context of the map and any unexplained or poorly fitted features were made obvious.

The use of computers as aids in the building of models started with Diamond's automatic procedures to fit a model to coordinates read from the map (*Acta Cryst.*, **21**, 253; 1966). This approach has been extended to the refinement of a model to fit an electron density map itself ("real-space" refinement), rather than guide coordinates (*Acta Cryst.*, **A27**, 436; 1971). Refinement is an essential part of the structure-solving process and Diesenhofer and Steigmann (*Acta Cryst.*, **B31**, 238; 1975) have taken Diamond's method farther by improving the phasing of the structure factors. They used the model fitted to a purely experimental electron density map to calculate structure factor phases for a new map. By repetition of this process the model and the phases were cyclically refined together.

Diamond and Levitt (*J. molec. Biol.*, **82**, 371 and 393; 1974) attacked another problem of the refinement of models, that of energy, both bonded and nonbonded, attractive and repulsive. In their work on the structure of lysozyme they alternated energy and real-space refinement finding that the alterations suggested by energy considerations tended to improve the fit to the electron density data.

This use of energy calculations in conjunction with refinement against an experimental map obviated one of the criticisms of those who advocated the building of an actual model and its improvement as the only way of introducing intramolecular and intermolecular interactions. Nevertheless the viewing of a model superimposed on an electron density map has been not only a precursor of refinement but also important itself. Even where methods of refinement have been used to extract as much information as possible from the diffraction data without recourse to model building, a model must be built to examine the biological implications of the structure.

The difficulties of constructing a very large Richard's box, of reading off the coordinates from one, of having either several boxes or being prepared rapidly to interchange models and density maps made the idea of a computer driven visual display system attractive Levinthal (*Sci. Amer.*, **214**(6) 42; 1966) started drawing on a visual display skeletal models of proteins which could be viewed from different angles or caused to rotate. The ability to alter or add to a computer drawn model and the simultaneous display of a three-dimensional density map has since been developed and in a number of laboratories (see for example *Fedn Proc.*, **33**, 2368: 1974 and *Science*, **190**, 1047; 1975).

Given a refinement system and a good catchnet to enable one to examine by eye those regions of the model which appeared to be problematical, the recent work by Greer (*J. molec. Biol.*, **100**, 427; 1976) would imply an ability to analyse completely an electron density map without ever actually looking at it in its entirety. One can envisage a protein structure so large that even with new display technology its map could not be viewed in one picture. The building of a model using the display system would require excessive searching across the boundaries of the displayed region. Preliminary automatic computer model building by Greer's method followed by examination using a stereoscopic visual display system would then provide a suitable preparation for refinement.

A large structure such as this would not be amenable to the building of a physical model after refinement. Studying the computer model would then also require a stereoscopic display system and techniques for searching through a large three-dimensional structure which can be seen, as it were, through a small window. Such a display system would facilitate comparison of structural results from other laboratories and the teaching of structural principles. It would, however, lack the presence of a good old-fashioned wire model. □

Contraception

from J. P. Hearn

A meeting on Contraceptives of the Future convened by R. V. Short and D. T. Baird was held by The Royal Society on February 18 and 19, 1976. The invited speakers examined the advantages, disadvantages and the problems of distribution of currently available methods for controlling fertility, and discussed new techniques that are being developed.

CONTRACEPTION is here to stay. In the past the natural checks on human populations were a relatively late age at puberty, high neonatal mortality, a long interval between births and a short life expectancy. Man has reversed all of these natural controls in most developed and developing societies. Menstruation now begins at about 11 years of age, neonatal mortality is very low, urbanisation and improvements in nutrition have more than halved the natural lactation-induced interval between births, and average life expectancy has almost doubled.

All these naturally occurring limiting factors were female orientated mechanisms, and since natural selection has always operated in the past to maximise reproductive potential, women may be physiologically ill adapted to spend the greater part of their reproductive lives in the non-pregnant state. As far as possible, new contraceptives should therefore be designed to mimic the physiological state found during periods of natural infertility in women. There are already many methods of contraception available but cultural, political and medical considerations make few of them universally acceptable so that there is a pressing need for new methods to be developed for both women and men. Ideally any new method should be long lasting, cheap, reversible, convenient and free from the need for continual medical supervision.

Expenditure on the development and application of contraceptives is only 2% of all the funds going to biomedical

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Archaean-Proterozoic polar wandering

from Peter J. Smith

THERE is now considerable geological and palaeomagnetic evidence to suggest that there were no large relative movements of plate segments during the Proterozoic. There was almost certainly some internal deformation along mobile zones between rigid cratons, although any relative motion such a process may have involved was evidently too small to be resolved by current palaeomagnetic methods. In any event, the palaeomagnetic data analysed by Piper *et al.* (*Nature*, **245**, 244; 1973) showed that for the period 2,000–800 million years ago the apparent polar wandering paths for reconstructed Africa and North America (the two most widely studied continents) were in close agreement.

But what about the period before 2,000 million years ago? Until recently it would have been difficult to answer this question because appropriate palaeomagnetic data were few and many more of those that were available were assigned unreliable ages. According to Piper (*Earth Planet. Sci. Lett.*, **28**, 470; 1976), however, this situation no longer obtains, at least for all of the period before 2,000 million years. Not only are there now a considerable number of relevant palaeomagnetic poles available but many of them have been well dated, particularly by the Rb-Sr method.

Because igneous activity during the Precambrian was episodic, some time intervals are better represented in the palaeomagnetic record than others. The two best to date are 2,160–2,000 million years and 2,700–2,500 million years. Piper concludes that throughout both of these periods the combined poles from Africa and North America lie on a single apparent polar wandering curve when the two continents are reconstructed on the basis of the younger Precambrian palaeomagnetic data. In short, the two continents apparently acted as a single unit, albeit with some internal deformation undetectable palaeomagnetically, throughout the whole period 2,700–800 million years ago.

research. The current level of funding, an annual $120 million, has been steadily reduced over the past three years by inflation. Most funding comes from the developed countries, where the lower birth rates now achieved may inhibit governments from future spending on contraceptive research. But research in reproduction may also pay off in the treatment of infertility, which is the other side of the same problem At present basic research accounts for 60% of the funds, the development and application of contraceptives takes 30%, and the remaining 10% goes into training personnel and into testing for safety. This latest component will have to be increased in the future as ethical and drug administrative regulations have become increasingly stringent. Investment in contraceptive research is essentially long-term, and seldom commercially rewarding. Pharmaceutical companies are unlikely to spend as much on contraceptive research in the future since testing on several animal species, including monkeys, makes it already a great deal more expensive to bring a new contraceptive on to the market than, for example, a new drug for the treatment of heart disease. The problems of regulating human fertility will not be solved in the near future and by the early 1980s the level of expenditure will need to be about $500 million each year.

Undoubtedly the most effective and acceptable contraceptive available is the combined gestogen–oestrogen pill. Although this method has a few disadvantages and does not suit all women, it will continue to be the most important contraceptive used over the next ten years in the developed and developing countries. The ways in which it is introduced and marketed in developing countries require a great deal more thought, adapting the approach to a wide variety of cultural differences. The pill causes infertility by creating the hormonal state normally found during pregnancy. It may be possible to develop other types of pill to mimic the hormonal states found during lactation or before puberty, the other naturally occurring periods of infertility in women Condoms are still probably the most widely used method in many countries and although they are not so reliable, their simplicity and lack of any disadvantageous effects makes them the method of choice for many people. Intra-uterine devices and hormonal implants still require a considerable amount of development before they are as effective or as acceptable. Abortion in early pregnancy is clearly effective, but requires medical or paramedical supervision and is not a contraceptive. In some countries abortion has been used widely but to many people it represents an unacceptable policy of despair, a consequence of the failure to produce and distribute adequate contraceptive technology.

There have been advances in the field of surgical sterilisation, particularly in the female, that hold promise for a simple, reversible method Developments in colpotomy, culdoscopy and laparoscopy have minimised the surgical procedures involved and markedly reduced the time required for recovery Attempts to use removable clips on the oviducts may lead to "temporary sterilisation" as a means for spacing a family rather than an irreversible technique to be applied only when the family is complete. In the male, sterilisation by vasectomy is widely used but as yet reversibility is not generally possible. There has been an interesting trend in that sterilisation is becoming more accepted amongst women and now as many women as men are being sterilised when they have completed their families.

The World Health Organization, in its expanded programmes of research in human reproduction, is supporting research projects aimed at improving most of the currently available methods of fertility control and also at developing new methods. WHO and other organisations, such as the Population Council and the Ford Foundation, are supporting research into more reliable ways of predicting ovulation and so using abstinence during the critical stages of the natural cycle as a method of birth control. This approach is complicated as the human sperm can survive in the female genital tract for up to seven days, but if ovulation could be predicted more precisely such methods may help those who find other methods unacceptable for cultural or religious reasons. Research is also going on to examine naturally occurring plant substances that have been used as contraceptives in the past in some societies.

It seems unlikely that any new method of fertility control that fulfils the requirements of being long lasting, cheap and reversible will be widely available in the next 5-10 years but two new concepts that may develop relatively rapidly are a pill for men and immunological control of fertility in men and women. Tests on small numbers of men have shown that a combined gestogen–androgen pill will suppress spermatogenesis and not interfere with libido, but the variation in response to this treatment is too wide at present and further tests are necessary to ensure its efficacy and to examine any possible toxicity. At present it seems likely that this method will be reversible.

Studies on marmoset monkeys have shown that it is possible to immunise primates against a fraction of chorionic gonadotropin, a hormone normally produced in the earliest stages of pregnancy which is thought to stimulate the production of progesterone from the ovary. Immunised marmosets will remain infertile for many months, but a great deal more research into possible side effects is necessary before this method can be applied In particular it is necessary to show that the antibodies do not cross react with endogenous hormones nor have any effects on foetuses as the contraceptive effects of the vaccine wear out. In the meantime research in India has shown that human chorionic gonadotropin can be made antigenic in women and in the United States researchers have made synthetic fractions of chorionic gonadotropin that may, in the long term, enable the production of a non cross-reacting and highly specific vaccine. Further work is in progress to try to make the vaccine reversible. The fact that human hormones can be made antigenic in the human opens up the immunological possibilities of fertility control, but any vaccines that are developed against hormones or enzymes involved in the development or capacitation of human gametes will need to act specifically and not upset other components of the system.

If we accept that from now on we shall be dependent on artificial forms of contraception then tomorrow's contraceptives must be chosen with great care, as they will have a considerable impact on our general health and social well being. ☐

Sickle cell disease

by Stuart J. Edelstein

A symposium on Molecular and Cellular Aspects of Sickle Cell Disease was held in Dallas on December 10, 1975, under the sponsorship of the Sickle Cell Disease Branch of the National Heart and Lung Institute and the University of Texas Health Science Center.

Sickle cell disease is a genetic abnormality that is understood in many details: a base change A→C in the DNA of the β-globin gene leads to the Glu→Val change at the β-6 positions of haemoglobin. The mutant form, designated haemoglobin S, has diminished solubility in the deoxygenated state and self-associates into the long, rigid fibres that distort the red cells into the characteristic sickle shape and are responsible for the various symptoms of the disease. An earlier hope that cyanate treatment would provide a suitable therapy for controlling sickling crises has faded and the search for an anti-sickling agent continues. Such an agent could be found by the trial-and-error screening of many compounds or by design based on the molecular details of fibre formation which are now emerging and which were summarised at the Dallas symposium.

The day began with B. C. Wishner (Johns Hopkins University, Baltimore) reporting on the structure of a crystalline form of haemoglobin S that may provide clues to the contacts between molecules in the fibres. In the crystals, molecules are arranged in paired strands, with adjacent strands within each pair staggered by half a molecule and rotated by half a turn An. intriguing feature of this crystal structure is that the β-6 position lies at an intermolecular contact between the strands within each pair, and several other sites implicated in the sickling process are also at or near contacts. If the axis of the strands in the crystals corresponds to the fibre axis *in vivo,* the orientation of the molecules in the crystals also satisfies critical optical dichroism data. Thus the juxtaposition of molecules in the crystals could bear a close resemblance to the arrangements in the fibres (with minor adjustments for converting the planar alignment of the crystals to the helical alignment of the fibres).

The possibility of a close relationship between the crystals and the fibres was strengthened by the presentation of S. J Edelstein (Cornell University, Ithaca) on the structure of the fibres determined by electron microscopy, in collaboration with R. Josephs (Weizmann Institute of Science, Rehovot). The fibres were prepared by lysis of sickled cells directly with solutions of negative stain, revealing eight-stranded fibres with adjacent strands staggered by half a molecule as the predominant form in all 10 individuals with sickle cell disease examined The structure of the fibres was deduced from optical diffraction and Fourier transform analysis of the electron micrographs and is consistent with an assembly of four pairs of strands in the general arrangement found in the crystals studied by Wishner *et al.* Uncertainties remain, including whether adjacent pairs of strands would be parallel or antiparallel and why the eight-stranded, staggered-strand structure was not detected in earlier studies by Finch *et al* (*Proc. natn. Acad. Sci. U S A* , **70,** 718; 1973)

On the basis of solubility studies of artificial hybrid haemoglobin constructed of β–S and various α-chain mutant forms, R Benesch (Columbia University. New York) reported five new α-chain positions apparently involved in sickling The two positions with the strongest influence on sickling, α47 and α54, were among the α-chain locations predicted to lie at contacts from the model building based on the electron microscopic results noted above. Thus several lines of investigation are converging on the structural details of the fibres, and a definitive description may soon emerge. While such information should help in the design of anti-sickling agents, there are also likely to be many physical-chemical subtleties in fibre assembly yet to be appreciated. One such subtlety emerged from the presentation of A. N. Schechter (National Institutes of Health, Bethesda) concerning a synthetic peptide corresponding to the first 13 residues of the β–S chain. The peptide binds to an antibody elicited in response to haemoglobin S, but does not itself influence fibre formation. Conformational differences between normal haemoglobin and S were also reported by C. Ho (University of Pittsburgh) on the basis of nuclear magnetic resonance studies. Therefore some aspects of conformation may be critical, along with other possible factors related to intermediate structures and rate limiting steps in fibre assembly.

The 11 other presentations dealt principally with aspects of fibre assembly using various techniques to monitor assembly, including an elegant application of water proton relaxation rates reported by M. R Waterman (University of Texas, Dallas). Various stages in the assembly process were described on the basis of viscosity by H F Bunn (Harvard Medical School, Boston) and ultracentrifugation by R W Briehl (Albert Einstein College of Medicine, New York). W A. Eaton (National Institutes of Health, Bethesda) presented a particularly cogent analysis of the delay time, t_d, for fibre formation, in relation to the "supersaturation ratio", S, raised to a power, n, equivalent to the order of the reaction:

$$1/t_d=\gamma S^n$$

where S is defined as the total concentration of haemoglobin S divided by the

Light in dielectrics

from a Correspondent

FOR the past sixty years there has been disagreement about the ratio of momentum to energy carried by electromagnetic waves in dielectrics. Abraham said $1/nc$; Minkowski said n/c, where c is the speed of light *in vacuo* and n the refractive index, so that c/n is the speed of light in the medium. The question relates to the correct choice and interpretation of the energy-momentum tensor. Subsequent contributors though relatively few have been exceptionally distinguished. This is doubly surprising. First, electromagnetic theory leaves no genuine scope for controversy; second, when the fog began to clear around 1973, the question was found to turn on a clear and detailed description, on the molecular level, of what precisely is measured in any given experiment. In other words the apparent simplicity of the problem as originally formulated proved deceptive.

Continuing the early and especially elegant discussion by Ginzburg (*Sov. Phys* (Uspekhi) **16,** 434; 1973) and Gordon (*Phys. Rev.,* **A8,** 14; 1973), further steps towards clarifying the issue have now been published by Sir Rudolf Peierls (*Proc. R Soc. Lond.,* **A347,** 475; 1976) Central to the issue is the distinction between the purely electromagnetic momentum (which by itself would lead to Abraham's result), and the mechanical momentum induced in the molecules of the medium. In an infinite amorphous (fluid) medium a broad enough light pulse leaves no mechanical disturbance in its wake; but on reflection from an interface it deposits mechanical stresses and strains just upstream of the interface, and these must be considered carefully in accounting for any observations. Professor Peierls has found that to leading order in (n^2-1), the operative result is the mean of Abraham's and Minkowski's To higher order in (n^2-1), there may be need to reflect further on this result, which, (as Peierls himself discusses), is *prima facie* in conflict with some arguments in the classic text by Landau and Lifshitz (*Electrodynamics of continuous media,* Pergamon Press, 1960). For narrow beams (with width to length ratio less than the ratio between the speed of sound and light), there are other effects, some of experimental importance considered already by Gordon (op. cit.), and another fascinating one found by Professor Peierls, reminiscent of Cerenkov radiation, of sound generation by the light pulse.

One thing that will surely be needed soon is a systematic textbook-level discussion explaining *ab initio* what results are applicable in what circumstances. For instance, a very recent low-frequency experiment on a solid dielectric (Walker *et al* , *Can. J. Phys* , **53,** 2577; 1975) is accounted for by Abraham's result alone, while in many quantum-mechanical applications Minkowski's result, alone, can be used; the "momentum" implied by the latter is then analogous to the so-called "crystal-momentum" of phonons.

minimum concentration for fibre formation and γ is a kinetic coefficient. Since red blood cells remain for about 1 s in capillaries, the delay time is the critical factor in whether or not a cell sickles and since the order is extremely high (about 40th), small changes in the parameters contributing to S can greatly alter the delay time. For example, the analysis indicates that the delay time in sickle cell trait is about 25,000 times longer than for sickle cell disease. Thus individuals with the trait are essentially free of symptoms and even a small increase in the delay time, as produced by some therapeutic agent, could greatly ameliorate the clinical course of the disease.

The sickling process is also complicated by factors related to the cell membrane. This point was emphasised in the presentation of M. J. Messer (University of California, San Francisco) who discussed experiments on cell sickling monitored by filterability, light microscopy and electron microscopy. When sickling is initiated by rapid deoxygenation, cell rigidity is detected before intracellular fibres or shape changes in the cells appear.

In summary, while considerable progress is being made in sickle cell disease, the complexities of a highly co-operative anisotropic assembly reaction in the confines of a cell membrane are likely to hold many surprises. The latest findings as reported at the Dallas symposium should soon be available to interested readers in detail, since the organisers promise prompt publication of the proceedings. □

Calculating radiation risks

from a Correspondent

A meeting organised by the Society for Radiological Protection was held in London on February 3.

What risks are you prepared to accept and what risks does your society seem prepared to accept? These were among the questions tackled by W. D. Rowe (US Environmental Protection Agency). The problem of assessing the biological effects of exposing large populations to small amounts of ionising radiation is currently a live issue in radiological protection circles. The man rem is the unit for the quantity 'collective dose equivalent' used in discussions of this question and the theme of the Society's meeting was the application of the man rem concept. Rowe pointed out that a central issue was the association of man rems with risk. The risks were to be balanced against the benefit which the population derived from the practices giving rise to the exposure. There were different types of risk, those arising from the planned releases of radioactive materials and those resulting from accidental situations. Rowe went on to analyse the factors important in the evaluation of risks and accepted that value judgments were an essential part of this procedure. The Environmental Protection Agency had, for example, used collective dose for regulatory purposes. They had set standards and determined the cost of carrying these out by using what Rowe called "operational value judgments" to determine how much money should be spent on "health effect avoidance". He felt that such value judgments were useful in this context for although subjective they did lead to decisions and action. They could be challenged and discussed and had been found to be helpful for that reason.

Any consideration of collective dose must deal with the significance of the large numbers of man rems arising from very small doses to very large numbers of people. P. M. Bryant (National Radiological Protection Board) had some forthright suggestions to offer. She felt that as we were all exposed to natural background radiation and as its level varied, for example, with geographical location, for the purpose of determining collective dose it was not reasonable to integrate doses below a level which fell well within the variation of natural background. She considered that it should be possible to define processes giving rise to exposures of the public in terms of "practices" which were of such a nature that significant numbers of individuals exposed in the overlapping fringe areas of several practices did not unwittingly receive more than the standard deviation of the background, that is, 10 millirems per year. A practice so defined might be, for example, all the long lived nuclides discharged during the 30 years of the life of a nuclear power plant. Bryant thought that if a reduction in the 10 millirem was made by one order of magnitude to allow for the number of practices going on at any one time and by another order of magnitude to allow for considerations of the future, then the resulting 0.1 mrem per year could be used as a cut-off. Below this the doses would be deemed to be insignificant and would not be used in any integration to obtain collective dose equivalent where such integration is intended for making choices between one practice and another. It seemed to her that this cut-off level would assist in realistic cost/benefit analysis although she admitted that if her concept of "collective dose from a practice" were used the definition of the practice might sometimes present difficulties.

During his discussion of two methods of considering radioactive releases arising from the operation of nuclear-powered electricity generating stations, R. H. Clarke (CEGB Berkeley Nuclear Laboratories) commented that the Canadians neglected doses below 1% of the dose at the site boundary and the Americans also employed a cut-off by integrating only the doses received within 50 miles of the site. Releases could be considered in terms of the doses given to a hypothetical critical group of the public located at the site boundary close to the release point. If the doses to this group were kept within the limits recommended by international authorities, then it followed that the doses received by the rest of the population would also be considered acceptable. This in fact was the approach used at present by the Central Electricity Board, but Clarke pointed out that it was possible for the doses to the critical group to remain within the arbitrary limits, yet, by varying stack heights, the distance from the release point to the site boundary and the distribution of the population around the site, the collective dose equivalent from similar releases of xenon-133 could vary by two orders of magnitude. This indicated a weakness of the critical group approach and in future although the doses to the most exposed individuals would remain important, some kind of limitation on the population collective dose around the site might be a more realistic parameter for the design engineer to aim at.

R. H. Mole (MRC Radiobiology Unit, Harwell) speaking in his private capacity, said it was important to distinguish between the validity of using man rems for thinking about exposures and for adding units of risk, and the validity of converting man rems into cases of detriment or hurt in some sense. Alternative hypotheses concerning the slope of the dose response relationship had to be considered. He was personally inclined to accept the linear hypothesis and was therefore critical of those who proposed a cut-off. It seemed to him that there was no adequate scientific reason for rejecting linearity and he suspected that there must be some "cultural" reason for distrusting it. Perhaps people were uneasy about calculating risks on the basis of a hypothesis which could not be demonstrated to be true.

The meeting was concluded by Mole pleading for those who published estimates of collective dose to set out every step of their calculations and in particular to state the range of uncertainty of the final number. A salutory exercise, no doubt! □

review article

Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters†

Paul Greengard*

The postsynaptic actions of some neurotransmitters may be mediated through cyclic nucleotides and cyclic nucleotide-dependent phosphorylation of specific membrane proteins in postsynaptic cells. In addition to providing a molecular basis for the actions of several neurotransmitters and of certain drugs affecting behaviour, the model suggests a mechanism by which neurotransmitter signals may be converted into electrophysiological responses in postsynaptic cells.

THE pioneer work of Sutherland, Rall and their colleagues firmly established that cyclic AMP acts as the intracellular mediator for the regulatory actions of many hormones on a variety of non-neuronal tissues[1]. There is more recent evidence that cyclic AMP also has an important role in the nervous system, where it seems to participate in certain types of communication between nerve cells. Cyclic AMP is implicated in at least three discrete processes in the nervous system, namely, mediation of the postsynaptic actions of certain neurotransmitters, regulation of the biosynthesis of certain neurotransmitters, and functioning of microtubules. Other evidence suggests that cyclic GMP is also involved in some types of synaptic transmission. This article summarises some of the evidence for a role of cyclic AMP and cyclic GMP as mediators of the postsynaptic actions of neurotransmitters and examines a possible mechanism for their action—regulation of the phosphorylation of specific proteins present in the postsynaptic membrane.

Cyclic nucleotides in synaptic transmission

Neurotransmitter molecules released from the terminals of the presynaptic axon induce a change in the membrane potential of the postsynaptic neurone by interacting with specific receptors on that neurone. This electrical change is referred to as the postsynaptic potential. How the activation of a postsynaptic receptor is transduced into the postsynaptic potential is still unknown; but it now seems that, in some instances, cyclic nucleotides are involved. Some of the experimental criteria that have been examined and found to support this view are presented in Table 1. Some of the neuronal systems for which a number of these criteria have been fulfilled are reviewed elsewhere[2].

One preparation that has been used to study the possible role of cyclic nucleotides in neuronal function is the mammalian superior cervical sympathetic ganglion. A diagram of the principal synaptic connections and the neurotransmitters believed[3–8] to occur within the superior cervical ganglion is included in Fig. 1, along with the postulated site of involvement of cyclic AMP and cyclic GMP in the production of the postsynaptic potentials. The postganglionic neurone in this ganglion receives three types of innervation. In the principal pathway innervating the ganglion, acetylcholine released from preganglionic terminals activates nicotinic cholinergic receptors on the surface of the postganglionic neurone, leading to the generation of a fast excitatory postsynaptic potential (f-e.p.s.p.). When several of these f-e.p.s.p.s summate, an action potential is generated and is propagated down the axon of the postganglionic neurone. There is evidence that generation of the f-e.p.s.p. does not involve a cyclic nucleotide. Indeed, since the f-e.p.s.p. is very rapid (of the order of a few ms), it seems *a priori* unlikely that its generation and termination would involve a series of enzymatic reactions of the type that seem to be associated with cyclic nucleotide-mediated processes.

There are two slow postsynaptic potentials which modulate the excitability of the postganglionic neurones: a prolonged hyperpolarisation or slow inhibitory postsynaptic potential (s-i.p.s.p.), and a prolonged depolarisation or slow excitatory postsynaptic potential (s-e.p.s.p.). In contrast to the generation of the f-e.p.s.p., the generation of these modulatory slow postsynaptic potentials does seem to involve cyclic nucleotides (Fig. 1). In one of the modulatory pathways, acetylcholine (ACh) released from preganglionic fibres activates a dopaminergic interneurone leading to the release of dopamine; the released dopamine activates a dopamine receptor on the surface of the postganglionic neurone leading, through a process which seems to involve cyclic AMP, to the generation of the s-i.p.s.p. In the other modulatory pathway, ACh released from preganglionic terminals activates muscarinic cholinergic receptors on the surface of the postganglionic neurone leading, through a process which seems to involve cyclic GMP, to the generation of the s-e.p.s.p.

Almost all of the criteria listed in Table 1 for mediation by a cyclic nucleotide of a postsynaptic potential have been fulfilled for the generation by cyclic AMP of the s-i.p.s.p. in the superior cervical ganglion. To cite just a few examples: (1) electrical stimulation of the preganglionic cholinergic fibres innervating the ganglion[9], or application of exogenous dopamine to ganglion slices[10], causes an increase in the cyclic AMP content of the ganglion (cytochemical studies indicate that this increase

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†Adapted from a lecture presented at the Sir Henry Dale Centennial Symposium in Cambridge, UK, September 18, 1975.

Table 1 Some criteria for mediation by a cyclic nucleotide of a postsynaptic potential*

a Synaptic activation and cyclic nucleotide levels

1. Electrical stimulation of the presynaptic input should increase tissue levels of the cyclic nucleotide.
2. This increase in cyclic nucleotide level should be antagonised by pharmacological agents that block the postsynaptic potential.
3. The increase should not occur when transmitter release is prevented with low Ca^{2+}/high Mg^{2+}.
4. The increase should not occur when the postsynaptic cells are antidromically activated.

b Neurotransmitters and cyclic nucleotide levels

1. Levels of the cyclic nucleotide should increase when the intact tissue or blocks or slices of the tissue are exposed to the neurotransmitter thought to be responsible for the postsynaptic potential of interest.
2. This increase should be antagonised by agents that block the neurotransmitter-induced changes of postsynaptic potential.

c Neurotransmitters and adenylate (or guanylate) cyclase

1. A neurotransmitter-sensitive adenylate (or guanylate) cyclase should be demonstrable in cell-free preparations of the tissue.
2. Activation by the neurotransmitter of this neurotransmitter-sensitive adenylate (or guanylate) cyclase should be blocked by the same antagonists as in (*a*2) and (*b*2).

d Cytochemistry

1. Cytochemical techniques should demonstrate that cyclic nucleotide levels increase in the appropriate postsynaptic cells in response to synaptic activation.
2. Similarly, cytochemical techniques should demonstrate that cyclic nucleotide levels increase in the appropriate postsynaptic cells in response to the appropriate neurotransmitter.

e Phosphodiesterase inhibitors

1. Phosphodiesterase inhibitors should further increase the elevated levels of cyclic nucleotides achieved by activating the synaptic pathway, or by applying the putative neurotransmitter.
2. Phosphodiesterase inhibitors should potentiate the effects on the postsynaptic potential that follow activation of the synaptic pathway, or application of the putative neurotransmitter.

f Application of cyclic nucleotides

1. The cyclic nucleotide should mimic the physiological effects of activating the synaptic pathway and of applying the putative neurotransmitter.

*Taken from Beam and Greengard[2].

in cyclic AMP occurs primarily or exclusively in the postganglionic neurone[11]); (2) a dopamine-sensitive adenylate cyclase is present in homogenates of the ganglion[10]; (3) exogenous cyclic AMP, applied to the ganglion, causes hyperpolarisation of the postganglionic neurones, thus mimicking the hyperpolarisation seen on either preganglionic stimulation (the s-i.p.s.p.) or application of dopamine[12]; (4) phosphodiesterase inhibitors, applied to the ganglion, greatly increase the elevation of cyclic AMP seen on preganglionic stimulation[8] or application of dopamine[13], and also greatly increase the size and duration, both of the s-i.p.s.p.[12] and of the dopamine-induced hyperpolarisation[12]; (5) various adrenergic and cholinergic agents affect the s-i.p.s.p.[3,5,6,12,14], as well as the increase in cyclic AMP which follows preganglionic stimulation[8], in a fashion predicted by the scheme shown in Fig. 1. Analogous data have been obtained for cyclic GMP in the generation of the s-e.p.s.p.[11–13,15].

These studies indicate that cyclic AMP mediates dopaminergic transmission, and cyclic GMP mediates muscarinic cholinergic transmission, in the superior cervical ganglion. They also suggest that the dopamine receptor of the postganglionic neurone is a dopamine-sensitive adenylate cyclase, the activation of which leads to an increase in cyclic AMP. Finally, cyclic AMP and cyclic GMP, through generation of the s-i.p.s.p. and s-e.p.s.p., respectively, seem to regulate the membrane potential of the postganglionic neurone, and thereby modulate nicotinic cholinergic transmission through the ganglion.

Extensive electrophysiological, pharmacological, and cytochemical studies by Siggins, Hoffer and Bloom, have provided good evidence that cyclic AMP mediates transmission at the noradrenergic synapse on the cerebellar Purkinje cell[16–19]. Stimulation of the noradrenergic fibres to the Purkinje cell reduces its firing rate, an effect mimicked both by noradrenaline and by cyclic AMP applied iontophoretically. The inhibitory effects of stimulating the noradrenergic fibres, or of applying noradrenaline or cyclic AMP, are markedly potentiated by phosphodiesterase inhibitors. Moreover, the inhibitory effects are associated, in each case, with hyperpolarisation of the Purkinje cell and a decrease in membrane conductance. These results indicate that the inhibitory effects of activity in this noradrenergic pathway are mediated through cyclic AMP.

Biochemical characterisation of dopamine receptors in mammalian brain

The studies of the superior cervical ganglion outlined above suggested a possible approach to understanding the molecular nature of dopamine receptors in the brain. Within recent years there has been, for several reasons, a great deal of interest in dopamine receptors in the central nervous system (CNS). There is increasing evidence for dopaminergic transmission within the brain[20–30] and aberrations of dopaminergic transmission may have a major role in the aetiology of some diseases of the CNS. For instance, hypoactivity of dopaminergic pathways to the caudate nucleus of the brain seems to be at least partly responsible for the symptoms of Parkinson's disease[21]. Conversely, hyperactivity of dopaminergic pathways to the limbic region of the brain may contribute to the symptoms of schizophrenia[31–34]. In addition, the parkinsonian side effects frequently seen in patients given antischizophrenic drugs, and possibly the therapeutic effects of these drugs as well, are largely attributable to their ability to block dopamine receptors in the brain[20,23,29]. Since the dopamine receptor in the superior cervical ganglion seemed to be the dopamine-binding portion of a dopamine-sensitive adenylate cyclase[10], and since a dopamine-sensitive adenylate cyclase was also found in retina[35], a search for such an enzyme was undertaken in the caudate nucleus of the brain, a region rich in dopamine receptors.

An adenylate cyclase, activated by low concentrations of dopamine, was found in homogenates of the caudate nucleus[36]. The enzyme was also activated by low concentrations of apomorphine, a known dopamine-receptor agonist. In contrast, isoproterenol, an effective agonist at β-adrenergic receptors, but not at dopamine receptors, failed, even in high concentrations, to stimulate the enzyme, indicating the specificity of the enzyme activation. Furthermore, the activation of the enzyme by dopamine was strongly inhibited by two classes of antischizophrenic compounds known to be dopamine-receptor antagonists. In contrast, several other pharmacological substances, with no antischizophrenic or dopamine-receptor antagonist properties, failed to block the dopamine activation of the enzyme. The results of this study indicated that the dopamine receptor in mammalian brain (as in the superior cervical ganglion) might be the dopamine-binding portion of a dopamine-sensitive adenylate cyclase, suggested that activation of this enzyme could account for the action of some antiparkinson drugs, and suggested a molecular basis for the actions of antischizophrenic drugs.

Subsequent investigations in several laboratories have strengthened and extended these conclusions[37–46]. An adenylate cyclase has been found in the limbic region of the brain[37,38,43] with characteristics virtually identical to those of the caudate enzyme. Moreover, in studies using a vast array of known agonists and antagonists of the dopamine receptor, the ability of substances to act as activators or inhibitors of the dopamine-sensitive adenylate cyclase system has shown, with few exceptions, a remarkable parallel with their ability to act as agonists or antagonists of the dopamine receptor[37–46].

As an illustration of such studies, Fig. 2 shows the effect of

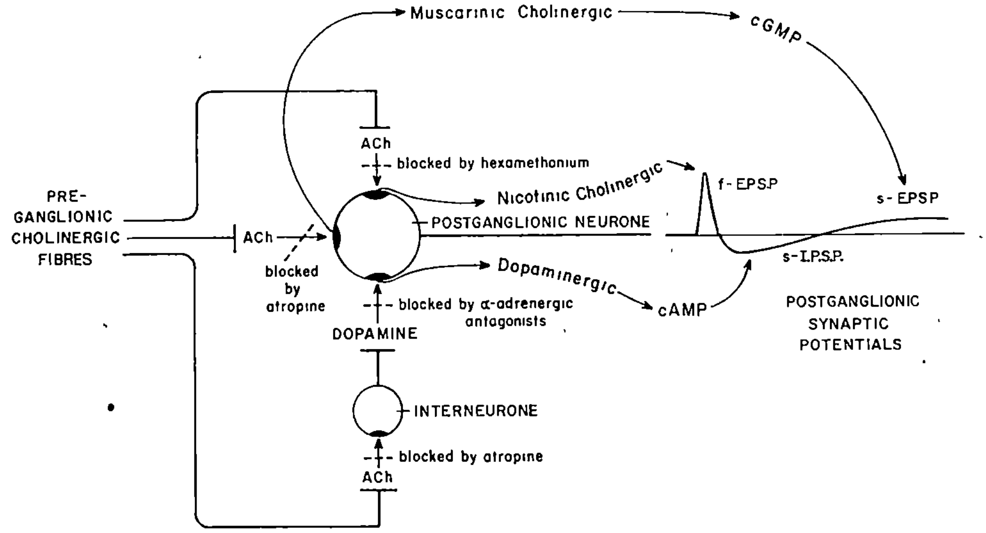


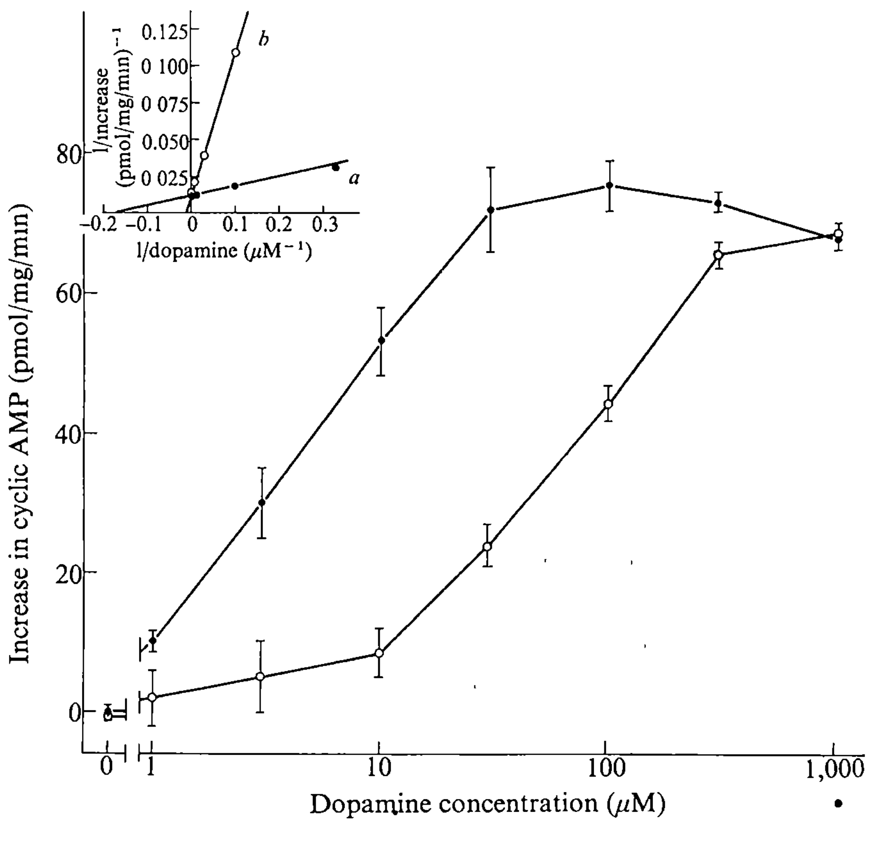
Fig. 1 A schematic diagram of the principal synaptic connections in the mammalian superior cervical ganglion, and the postulated role of cyclic nucleotides in the genesis of the postganglionic synaptic potentials. The diagram shows the relationship between the various neuronal elements; the neurotransmitters released at the various synapses; the sensitivity of the synaptic receptors to different classes of specific antagonists; the electrical signs that accompany activation of the various postganglionic receptors following preganglionic stimulation; and the postulated involvement of cyclic nucleotides in the production of the electrophysiological responses. Abbreviations used: ACh, acetylcholine; f-e.p.s.p., fast excitatory postsynaptic potential; s-i.p.s.p., slow inhibitory postsynaptic potential; s-e.p.s.p., slow excitatory postsynaptic potential. (Based on studies reviewed by Eccles and Libet[3], Volle[4], Libet[5], Greengard and McAfee[6], and Greengard and Kebabian[7]. Modified from Kalix *et al.*[8].)

dopamine and of fluphenazine (a potent antischizophrenic drug and dopamine-receptor antagonist) on the adenylate cyclase activity of a subcellular fraction, rich in synaptic membranes, from the rat caudate nucleus. Dopamine caused a half-maximal increase in enzyme activity at about 4×10^{-6} M, which was competitively inhibited by fluphenazine. Moreover, fluphenazine was extremely potent as an inhibitor of the enzyme, with an inhibition constant (K_i) of 8×10^{-9} M (ref. 46). The inhibition of the enzyme by fluphenazine and related compounds occurred at concentrations two to three orders of magnitude less than those at which most other effects of these substances have been found.

These studies support the conclusion that the dopamine receptor, in at least some regions of the brain, as in the superior cervical ganglion, is part of a dopamine-sensitive adenylate cyclase. A corollary of this conclusion is that cyclic AMP mediates synaptic transmission at such dopaminergic synapses.

Finally, these studies indicate that inhibition by the antischizophrenic drugs of dopamine-sensitive adenylate cyclase in the caudate nucleus may represent the molecular basis for the

Fig. 2 Effect of various concentrations of dopamine, in the absence (●) or presence (○) of 1×10^{-7} M fluphenazine, on adenylate cyclase activity in a particulate fraction of rat caudate nucleus rich in synaptic membranes. In the absence of added dopamine or fluphenazine, 48.1 pmol of cyclic AMP were formed per mg wet weight of tissue per min; in the presence of 0.1 μM fluphenazine, 47.9 pmol of cyclic AMP were formed. The increase in cyclic AMP above basal level (that is, the level in the absence of dopamine or fluphenazine) is plotted as a function of dopamine concentration. Inset: double-reciprocal plot of cyclic AMP increase as a function of dopamine concentration from 3 to 300 μM. *a*, Control; *b*, 1×10^{-7} M fluphenazine. (Taken from Clement-Cormier *et al.*[46].)



parkinsonian side effects of these compounds, and suggest that a similar inhibitory action on the enzyme in the limbic region may be responsible for the therapeutic effects of these drugs.

The evidence that a cyclic nucleotide mediates synaptic transmission is perhaps strongest in the case of dopaminergic synapses, due in part to the large variety of known agonists and antagonists of the dopamine receptor available for study. Nevertheless, cyclic AMP has been implicated in the postsynaptic response to several other putative neurotransmitters, including noradrenaline (acting at β-adrenergic receptors), 5-hydroxytryptamine (5-HT), octopamine, and histamine [47–53]. Moreover, the actions of several drugs that affect behaviour, including LSD[54,55], morphine[56–58], and the antischizophrenic drugs discussed above, may be attributable to actions on specific neurotransmitter-sensitive adenylate cyclases. Finally, studies of the superior cervical ganglion[11–13,15] and of brain[59–61] suggest, in the case of one neurotransmitter, namely ACh (acting at muscarinic cholinergic synapses), that cyclic GMP may act as a mediator of synaptic transmission. These various results raise the question of the nature of the receptors for the cyclic nucleotides in the postsynaptic cells.

Phosphorylated proteins in synaptic membranes

The studies summarised in this section have been carried out within the framework of the hypothesis[62] that the diverse effects of cyclic AMP in various tissues are mediated through regulation of the activity of a family of enzymes known as protein kinases. This class of enzymes catalyses the phosphorylation of substrate proteins by ATP. The protein kinase hypothesis was proposed after the discovery of cyclic AMP-dependent protein kinases, first in muscle[63], and then in liver[64] and brain[65] and explains how cyclic AMP, a small molecule, may exert the large variety of biological effects attributable to it. According to this hypothesis, cyclic AMP need only regulate the activity of a single class of enzymes, the protein kinases; the specificity of the cyclic AMP action is then accounted for in terms of the specificity of the protein kinases and, particularly, of the substrate proteins which are phosphorylated by these protein kinases, in the various tissues.

The application of the protein kinase hypothesis to synaptic transmission in the nervous system is illustrated in Fig. 3. Three distinct roles of cyclic AMP and protein phosphorylation in neuronal function, for which there is now evidence, are illustrated. Two of these roles fall outside the main theme of this presentation, and will be described only briefly.

First, cyclic AMP-dependent protein kinase seems to be capable of regulating the biosynthesis of neurotransmitters in presynaptic terminals. Cyclic AMP has been shown to activate tyrosine hydroxylase[66–69], the enzyme that controls the rate of biosynthesis of the catecholamines dopamine and noradrenaline. The activation by cyclic AMP of tyrosine hydroxylase is almost certainly mediated through a protein kinase, since the activation by the cyclic nucleotide (1) requires ATP and Mg^{2+} (refs 70–73), (2) is mimicked by a purified preparation of protein kinase[70], and (3) is abolished by various specific and nonspecific inhibitors of brain protein kinase[70]. Future studies may demonstrate that the rate of biosynthesis of neurotransmitters other than the catecholamines is also subject to regulation by cyclic AMP-dependent protein kinases.

Second, cyclic AMP and protein kinase in nervous tissue may influence the microtubule system. The possibility that cyclic AMP-dependent protein phosphorylation affects microtubule function was first suggested by Goodman *et al.*[74]. Recently, evidence has been obtained[75] that highly purified microtubules from chick brain contain an intrinsic cyclic AMP-dependent protein kinase which catalyses the stoichiometric phosphorylation of a substrate protein (with an apparent molecular weight of 300,000) that is also an integral component of the microtubule system. Experiments are in progress, using *in vitro* systems, to determine whether the cyclic AMP-dependent phosphorylation of this high molecular weight protein affects some parameter of microtubule function, such

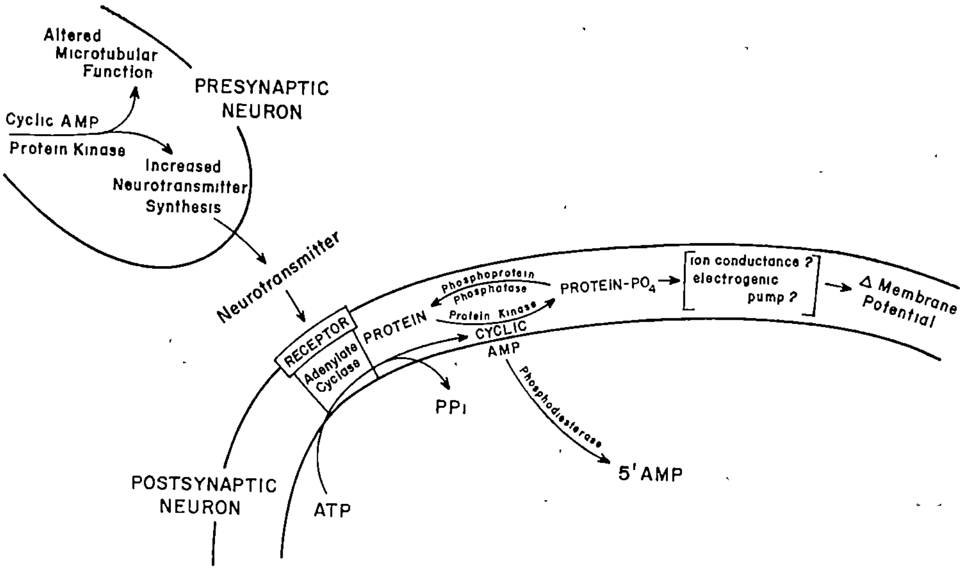


Fig. 3 Proposed roles for cyclic AMP and protein phosphorylation in neuronal function include regulation of neurotransmitter synthesis in presynaptic terminals (*a*), regulation of microtubular function (*b*) (indicated, purely for convenience, as occurring in the presynaptic axon; microtubules occur in dendrites, soma and axon of neurones), and generation of postsynaptic potentials (*c*). The sequence of events by which neurotransmitter, released from presynaptic terminals, produces an electrophysiological response in the postsynaptic cell is conceived as follows. The released neurotransmitter activates a neurotransmitter-sensitive adenylate cyclase present in the membrane of the postsynaptic cell, leading to the production of cyclic AMP in the immediate vicinity of the postsynaptic membrane. The newly formed cyclic AMP activates a cyclic AMP-dependent protein kinase present in the postsynaptic membrane This activated protein kinase catalyses the phosphorylation of a substrate protein, also present in the postsynaptic membrane, converting it from the non-phosphorylated to the phosphorylated state. A key element of this model is that this substrate protein controls the permeability of the postsynaptic membrane. Phosphorylation of the substrate protein leads, through a change either in ion conductance or in the rate of an electrogenic pump, to a change in membrane potential, the "postsynaptic potential". Since the postsynaptic potential is transient in nature, enzymatic machinery must exist which terminates this sequence of events. This enzymatic machinery includes a phosphodiesterase that hydrolyses the cyclic AMP to 5'-AMP and a phosphoprotein phosphatase that converts the substrate protein back to the non-phosphorylated form, leading to the termination of the postsynaptic potential.

as the rate at which these microtubules transport material along their length, or the rate of assembly or disassembly of the microtubules.

The third possible role, which is the main theme of this presentation, is in mediating the postsynaptic effects of neurotransmitters at certain types of synapse. An hypothetical sequence of events by which this may occur is presented in Fig. 3. Although the scheme is far from proven, a variety of evidence is compatible with the idea that the effects of cyclic AMP on the membrane properties of postsynaptic cells may be mediated through regulation of the state of phosphorylation of specific membrane proteins. I shall now summarise some of the evidence bearing on this issue.

The five proteins indicated in the postsynaptic neurone in Fig. 3, namely adenylate cyclase[76], phosphodiesterase[76–78], protein kinase[79], protein kinase substrate[80], and phosphoprotein phosphatase[81], are present in very high concentrations in those subcellular fractions of brain tissue that are richest in synaptic membranes. Cyclic AMP markedly stimulates the phosphorylation, by endogenous protein kinase, of two substrate proteins, designated protein I and protein II, present in these synaptic membrane fractions (Fig. 4)[82]. The phosphorylation of both proteins reaches a maximum within 5 s of incubation, the shortest reaction time that has been studied. Since the molecular events underlying synaptic transmission must be rapid, the rapid phosphorylation of these proteins is almost a prerequisite to considering seriously the possibility that one or another of them might be involved in the generation of postsynaptic potentials. Studies of these synaptic membrane proteins have yielded some provocative results.

Studies of the subcellular distribution of protein I indicate large amounts are present in synaptic membranes[82]. In contrast, protein I has not been found in any subcellular fraction of any non-nervous tissue examined[82]. Two other studies of protein I, now in progress in our laboratory, can be mentioned briefly. First, Suzanne Lohmann has obtained evidence that the amount of protein I is very low in neonatal rat cerebrum, and that it increases sharply 2–3 weeks postnatally, when there is also a marked increase in histochemically demonstrable synaptic structures[83]. Secondly, Tetsufumi Ueda has succeeded in solubilising protein I from a synaptic membrane fraction of bovine brain, and has purified it approximately 1,000-fold, calculated with reference to the solubilised extract. Starting with 1.3 kg of brain, he has obtained about 900 µg of apparently pure protein I. It should be possible to collect enough protein I to carry out a study of its structure and of its physicochemical properties, and to determine whether phosphorylation alters these properties. Such studies could provide clues to the chemical basis for the physiological changes observed in some postsynaptic membranes during synaptic transmission. Purified protein I, in its non-phosphorylated and phosphorylated forms, could also be added to model membranes to see if either form affects their permeability.

Moreover, antibodies to purified protein I are now being prepared with several aims in mind, including developing a sensitive radioimmunoassay for protein I, examining the possible effect of the antibodies on neuronal function, and determining the cytochemical distribution of protein I. It will be of interest to see whether such an immunocytochemical approach verifies the localisation of protein I in the postsynaptic membrane.

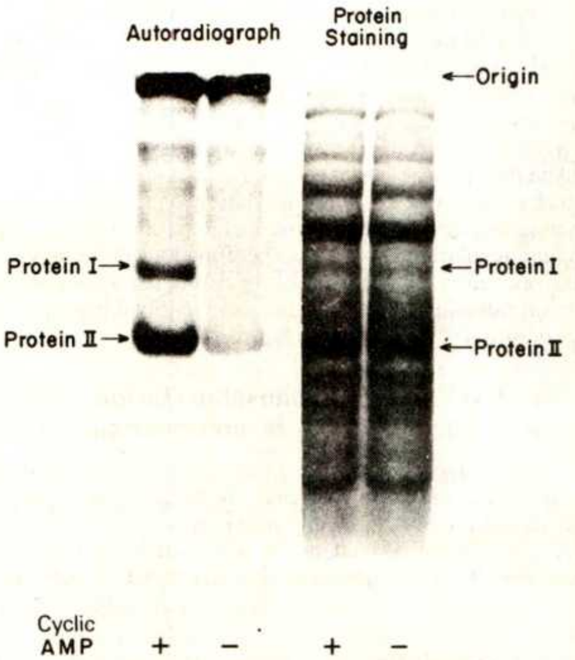


Fig. 4 Effect of cyclic AMP on the phosphorylation of endogenous membrane proteins in a synaptic membrane preparation from rat cerebrum. The synaptic membrane preparation was incubated with γ-^{32}P-ATP, in the absence or presence of cyclic AMP, for 15 s. The reaction was stopped by the addition of sodium dodecyl sulphate. The solubilised protein was then subjected to slab gel electrophoresis, protein staining, and autoradiography, to determine those protein bands into which radioactive phosphate had been incorporated. Autoradiograph is shown on the left side, and a photograph of the protein staining pattern of the dried gel is shown on the right side. The + and − signs at the bottom of the gel indicate the presence and absence, respectively, of 5×10^{-6} M cyclic AMP in the incubation mixture. Several dozen protein bands are visible in the protein staining pattern. A few of these bands incorporated radioactive phosphate, as indicated by the autoradiograph. Phosphorylation of two protein bands was markedly affected by cyclic AMP. The arrows identify the positions on the gel of these two protein bands, designated protein I and protein II, the apparent molecular weights of which are 86,000 and 48,000, respectively. (Taken from Ueda *et al.*[82].)

In contrast to the apparently unique localisation of protein I in neuronal tissue, protein II, or at least a protein with a number of properties similar to those of protein II, is present in membranes and cytosol of a variety of tissues. Certain properties of the protein II system suggest that it could be involved in molecular events underlying a variety of rapid physiological processes. For instance, the rate of phosphorylation and the rate of dephosphorylation of protein II are both stimulated by cyclic AMP[84]. Such a mechanism, while thermodynamically unappealing since the overall effect is to hydrolyse ATP, has the asset of providing a mechanism for a very short-lived pulse of phosphorylated protein. Recently, the protein II kinase–protein II–protein II phosphatase system has been solubilised from synaptic membranes[85]. A kinetic study indicates that the three proteins are present in the solubilised system, and presumably are present in the intact synaptic membranes, in the form of a complex. The existence of the three protein components of a protein phosphorylation–dephosphorylation system in the form of a complex in the synaptic membrane would overcome one theoretical problem associated with the idea that phosphorylation of membrane proteins may mediate the actions of some neurotransmitters. Thus, if the three proteins were separate from one another, and it were necessary to rely on diffusion for the substrate protein to interact with its kinase and phosphatase, the reaction rates might be too slow to underly events as rapid as those associated with the generation of a postsynaptic potential. By being in the form of a complex, the system is not limited by rates of diffusion and, therefore, the phosphorylation and dephosphorylation steps could be extremely rapid. Studies are in progress to determine whether the protein I system also exists as a complex in synaptic membranes.

It would be helpful, in evaluating the scheme shown in Fig. 3, to determine whether a correlation exists, in an appropriate intact neuronal preparation, between the time course of the phosphorylation and dephosphorylation of some membrane protein and the time course of the rise and decay of the postsynaptic potential. But the electrophysiological and the biochemical responses in neuronal tissue are so rapid that it is not feasible to carry out such a study at present. Cyclic nucleotides also are involved in regulating membrane function in many non-neuronal tissues. Fortunately, certain of these non-neuronal preparations have been found useful for testing the hypothesis that the demonstrated ability of cyclic AMP to regulate the permeability of cell membranes is mediated through control of the state of phosphorylation of specific membrane proteins. The results have proved of interest both for understanding regulation of membrane function in non-neuronal tissues, as well as for their possible relevance to an understanding of brain function. Some of the results obtained with such preparations will be summarised next.

Cyclic AMP, protein phosphorylation, and membrane permeability in non-neuronal systems

β-Adrenergic agonists, including isoproterenol, adrenaline, and noradrenaline, cause an increase in sodium and potassium fluxes across the plasma membranes of avian erythrocytes[86–88]. There is evidence[87,88] that cyclic AMP mediates this physiological response: β-adrenergic agonists increase the concentration of cyclic AMP in these cells and cyclic AMP mimics the effect of the β-adrenergic agents in increasing cation fluxes. Moreover, the response is slow enough to permit measurements of protein phosphorylation at various times in the course of the response. Experiments were therefore carried out with intact turkey erythrocytes preincubated with radioactive inorganic phosphate, to label the intracellular ATP pool, and then incubated in the presence of various test substances[89]. Exposure of intact turkey erythrocytes to β-adrenergic agonists for a period of a few minutes markedly affected the degree of phosphorylation of only one protein. This protein had an apparent molecular weight of 240,000 and was located in the plasma membrane. In a variety of experimental conditions, there was a good correlation between the phosphorylation of this membrane protein and the cation flux across the membrane.

The toad bladder is another preparation which has been used to explore the possibility that cyclic AMP may regulate membrane permeability by controlling the state of phosphorylation of a specific protein. Antidiuretic hormone (ADH) increases the sodium permeability of the apical membrane of toad bladder epithelium by a mechanism which seems to involve cyclic AMP. Thus, ADH causes an increase in the concentration of cyclic AMP in this epithelium and cyclic AMP mimics the effect of ADH in increasing sodium transport[90]. Using intact epithelial cells, a correlation has been found between the degree of phosphorylation of a specific protein, called protein D, and the rate of sodium transport, in a variety of experimental conditions in which the cyclic AMP levels were manipulated[91,92]. In addition, aldosterone, which causes a delayed increase in sodium transport across toad bladder epithelium, but apparently not by altering the concentration of cyclic AMP, also affects the degree of phosphorylation of protein D[93]. The effects of aldosterone, both on protein D phosphorylation and on sodium permeability, require *de novo* RNA and protein synthesis, whereas the corresponding effects of ADH, mediated through cyclic AMP, do not. Thus, both ADH and aldosterone regulate the sodium permeability of this tissue and also effect the phosphorylation of the same specific protein, although by different mechanisms.

Studies of a membrane fraction derived from the sarcoplasmic reticulum of myocardial cells have provided evidence that phosphorylation of a specific protein may be involved in regulation by cyclic AMP of Ca^{2+} transport across intracellular membranes in the heart[94–96]. The sarcoplasmic reticulum is believed to represent an uptake system for removing Ca^{2+} from the vicinity of the contractile components of muscle fibres. Cyclic AMP stimulates the active transport of Ca^{2+} into isolated vesicles derived from the sarcoplasmic reticulum. This effect of cyclic AMP may be a component of the mechanism by which β-adrenergic agonists shorten the duration of cardiac contraction. On addition of cyclic AMP to these isolated vesicles, a correlation is observed between increased rate of Ca^{2+} transport, stimulation of a Ca^{2+}–Mg^{2+}-dependent ATPase, and phosphorylation of a 22,000-dalton membrane protein[94–96]. This phosphorylated protein may act as a modulator of the Ca^{2+}–Mg^{2+} ATPase[95,96].

The results obtained with several biological systems studied to date are compatible with the proposal that regulation of the phosphorylation of specific membrane proteins is involved in the mechanism by which cyclic AMP alters ion transport across membranes. It must be emphasised however, that what has been demonstrated is correlation but not causality. The development of highly specific activators and inhibitors of membrane-bound protein kinases and protein phosphatases would be of substantial help in evaluating critically the nature of the relationship between protein phosphorylation and membrane permeability.

Cyclic GMP-dependent protein phosphorylation in smooth muscle

Recent studies with mammalian smooth muscle suggest that the effects of cyclic GMP on postsynaptic membranes, like those of cyclic AMP, may be mediated through regulation of the phosphorylation of specific membrane proteins. ACh, acting on muscarinic receptors, causes a rapid and large increase in cyclic GMP in smooth muscle[97,98]. A protein kinase specifically activated by low concentrations of cyclic GMP, rather than cyclic AMP, as well as endogenous substrate proteins for this cyclic GMP-dependent protein kinase, have been found in a membrane fraction from mammalian smooth muscle[99]. As shown in Fig. 5, a half-maximal increase in the degree of phosphorylation of two substrate proteins, designated protein GI and protein GII, catalysed by an endogenous protein kinase, occurred at about 4×10^{-8} M cyclic GMP, in a membrane fraction from the ductus deferens. A 10- to 20-fold higher concentration of cyclic AMP was required to stimulate phosphorylation of these membrane proteins to the same extent. Cyclic GMP-dependent phosphorylation of proteins GI and GII has been found in a membrane fraction from all types of smooth muscle examined[99]. In contrast, proteins GI and GII have not been found in the cell sap of smooth muscle nor have they been found in any particulate or soluble fraction of a number of other tissues examined. Thus, it would seem that proteins GI and GII are specific to certain membranes from smooth muscle. The fact that ACh increases the level of cyclic GMP in smooth muscle, and that low concentrations of cyclic GMP stimulate the phosphorylation of two proteins which seem to be specific to smooth muscle membranes, suggests that cyclic GMP, as well as proteins GI and GII, may be involved in mediating or modulating some of the physiological effects of ACh on smooth muscle.

Although cyclic GMP-dependent protein kinases were discovered several years ago[100–102], and have been demonstrated in a variety of tissues throughout the animal kingdom[100–106] including mammalian brain[101,103–105], smooth muscle is the only tissue in which any endogenous substrate proteins for this class of enzymes have yet been found. Demonstration of cyclic GMP-dependent phosphorylation of endogenous substrate proteins in neuronal tissue, by endogenous protein kinases, would be of considerable help in understanding the role and mechanism of action of cyclic GMP in the nervous system.

Multiple roles for cyclic nucleotides and protein phosphorylation in neuronal function

The data summarised in this article suggest that the postsynaptic actions of a number of neurotransmitters may be mediated through cyclic AMP or cyclic GMP. Moreover, it is proposed that the immediate targets of cyclic AMP and cyclic GMP are protein kinases whose activation results in the phosphorylation of specific proteins in the membranes of postsynaptic cells, and that the alteration of the degree of

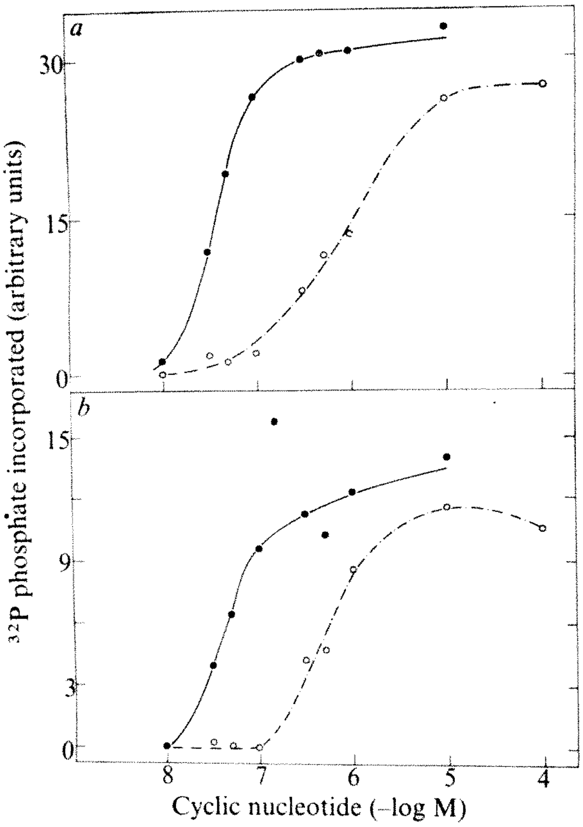


Fig. 5 Effect of various concentrations of cyclic GMP and cyclic AMP on the endogenous phosphorylation of proteins in a membrane preparation from guinea pig ductus deferens. The membrane preparation was incubated for 15 s with γ-^{32}P-ATP in the absence or presence of cyclic GMP (●; $K_a = 4 \times 10^{-8}$ M (*a*) or 5×10^{-8} M (*b*)) or cyclic AMP (○; $K_a = 8 \times 10^{-7}$ M (*a*) or 5×10^{-7} M (*b*)). The reaction was stopped by the addition of sodium dodecyl sulphate, and the solubilised protein was subjected to slab gel electrophoresis and autoradiography. Cyclic GMP markedly affected the phosphorylation of two protein bands, designated protein GI (*a*) and protein GII (*b*), with apparent molecular weights of 130,000 and 100,000, respectively. Incorporation of radioactive phosphate into these proteins was measured quantitatively by densitometric analysis of the autoradiograms. Results of those measurements are shown here. The data represent the increase in phosphorylation above that observed in the absence of added cyclic nucleotide, which amounted to 9.8 and 1.4 arbitrary units for protein GI and protein GII, respectively. (Taken from Casnellie and Greengard[99].)

phosphorylation of these membrane proteins produces the observed physiological effects of the neurotransmitters. It should, however, be emphasised that cyclic nucleotides and phosphorylated proteins may have many other roles in the nervous system. Two such roles include regulation of neurotransmitter synthesis and microtubular function, as discussed briefly above. The increase in cyclic AMP during nervous activity may also serve to mobilise carbohydrate and lipid reserves and thus to meet the increased energy demands associated with elevated functional activity. Cyclic AMP formed in response to neurotransmitters probably induces the synthesis *de novo* of a number of proteins with specific functional roles, including tyrosine hydroxylase[107–109] and *N*-acetyl transferase[110,111]. Finally, a 5-HT-mediated increase in cyclic AMP may be responsible for the increased neurotransmitter release that accompanies behavioural sensitisation in certain invertebrate nerve networks[112,113].

The biochemical mechanisms by which phenomena as varied as energy mobilisation, axonal transport, synaptic transmission, synthesis and activation of enzymes controlling neurotransmitter formation, and short term and long term information storage and retrieval are regulated in an orderly and coordinated fashion represent a challenging topic for future research in neurobiology. It seems likely that answers to many of the questions in this area will be found through understanding the regulatory and integrative role of cyclic nucleotides and phosphorylated proteins in the nervous system.

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articles

Laboratory investigation of tilt and seismicity anomalies in rock before failure

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Laboratory measurements of tilt and seismicity anomalies whose time durations are a few milliseconds are shown to precede failure in dry rock specimens. The observed tilt and seismicity behaviour is qualitatively similar to that reported to precede earthquakes. Test results suggest that this precursor time for laboratory specimens of this length may be independent of rock type, in agreement with theoretical predictions and other experimental data on rock failures including earthquakes.

A SCALE invariant theory of rock failure has been proposed that predicts precursor effects such as anomalous V_p, V_s and V_p/V_s behaviour and tilt before failure in rock[2]. In addition, because the theory does not vary with scale, these effects are predicted to occur on the laboratory scale over a time interval of a few milliseconds, and that this time interval will be independent of rock type[2,4]. Experimental evidence is presented below that indicates that tilt and seismicity precursors, whose characteristics are qualitatively similar to those observed before earthquakes, are observed a few milliseconds before failure of rock on the laboratory scale. Preliminary experimental results suggest this time interval may be independent of rock type.

Experimental procedure

Cylindrical samples of 5.39 cm diameter of a variety of rock types including Barre granite and Berea sandstone (reported here), oil shale, schist and marble were drilled, cut and ground to a final length of 13.5 cm. All specimens were deformed to failure under a confining pressure of 1.5×10^7 Pa (10^5 Pa = 1 bar) in a servo-controlled materials testing system. Tests were performed under deformation control with a displacement rate of 6.5×10^{-4} cm s^{-1}.

The load cell used in the testing programme was unique as both the x- and y-moments (M_x and M_y, respectively), in addition to the total load, F (z axis is load direction), could be measured and recorded on separate channels as a function of time. Bending moments, similar to tilts measured in regions prone to earthquakes, are measured about two perpendicular axes intersecting at the centre of the transducer and normal to the load axis. Moment and load changes were recorded on a visicorder and/or a digital memory scope (details of this equipment are available elsewhere[3]).

Acoustic emission, or equivalently, seismicity from the test samples was measured with a system (Dunegan/Endevco 3000) that included a log convertor (model 60) and a reset clock (model 402). The reset clock was modified to provide precision tuning pulses with a minimum pulse interval of 200 μs. The log converter produces a logarithmic scaling of both total emission and emission rate. A low mass ($\lesssim 1$ g) piezoelectric transducer with a ring-down time of ~ 20 μs was used to detect seismic emission from the test samples.

Experimental data

Figure 1 illustrates the variation with time of bending moment (M_x and M_y) for Barre granite (BG-T28) and Berea sandstone (BSS-5). Visicorder output of M_x and M_y as a function of time is shown in Fig. 1*b*. Photographs of both the time variation of

M_x and M_y for the first fracture in Berea sandstone (recorded on the digital oscilloscope) and the fractured test samples are shown in Fig. 1*a* and *c*, respectively. Note that the failure of the Barre granite specimen was a multiple (2) fracture event with the second fracture developing ~ 85 ms after the initial break. The failure of the Berea sandstone specimen, on the other hand, was a single fracture event. This observation should not be construed as suggesting that the existence of multiple or single fractures in failed rock is a function of rock type. Based on our tests, the occurrence of multiple compared with single fractures in failed rock depends only on such parameters as confining pressure, uniformity of loading and the existence of local inhomogeneities within the specimen. Moment and total load (F) variation with time for Barre granite specimens T14, T29 and T30 are displayed in the first column of Fig. 2*a–c*. Specimens T14 (Fig. 2*a*) and T30 (Fig. 2*c*) are single fracture events. Specimen T29 was a multiple (2) event. Moment and load changes for the first fracture in T29 are shown in Fig. 2*b*2 and *b*3.

The most prominent feature in both Figs 1 and 2 is tilting in the direction of eventual fault growth a few milliseconds before failure. This is followed by a reversal in tilt direction that culminates in specimen failure. The inferred initiation locations for failure (based on these moment changes) were substantiated by microscopic examination that showed increased crack concentrations in these locations (Fig. 1*c*). Note also that the total load in samples T29 and T30 decreases during this interval: the magnitudes of both the load decrease and moment changes within these specimens during this interval are ~ 2,500 N and 100 Nm, respectively. Also, the times of the tilt and load change anomalies are nearly identical. The tilt anomaly is, however, observed always to precede the load change, in this case, load decrease, by a time that is usually < 1 ms. Since the load changes within the specimen occur over such a small time, and because the primary hydraulic loading system of the machine cannot respond to deformation changes in $\lesssim$ 100 ms, load change within the specimen is transferred to the columns of the test machine. Thus, the sample continues to shorten during this time. It is not difficult to show theoretically that in these conditions the post-failure stiffness of the sample decreases during the anomaly time interval. Catastrophic failure occurs only when the post-failure stiffness of the sample equals the loading system stiffness[3].

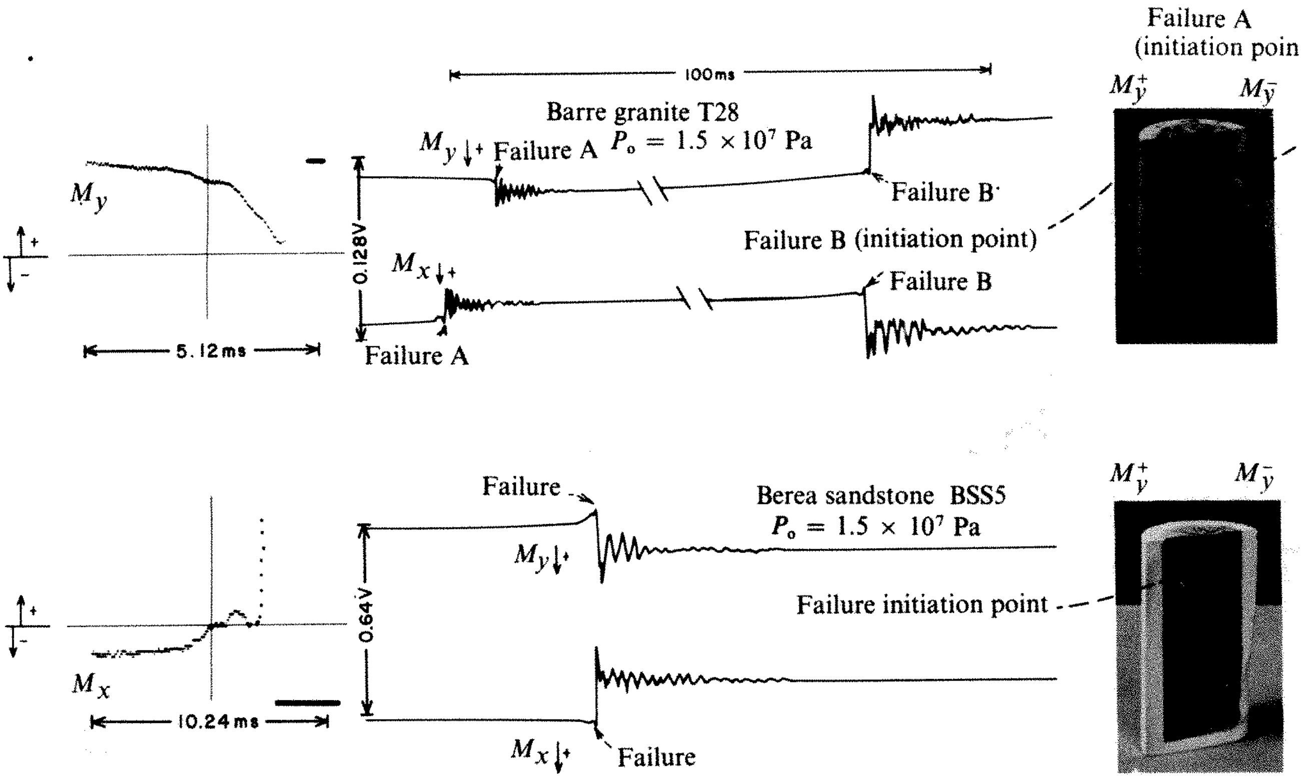


Fig. 1 Variation of M_y and M_x with time for Barre granite (BG-T28) and Berea sandstone (BSS-5). *a*, Photographs of digital oscilloscope output for moment variation. The conversion factor for the vertical scale (in volts) is 3.46×10^2 N m V^{-1}. *b*, Visicorder output of M_y and M_x against time; *c*, photographs of fractured test samples showing major fault zones.

Figure 3 displays moment (M_y) and acoustic emission variation [$N(t)$] with time for Barre granite specimens BG T23 (Fig. 3*a–c*) and BG T24 (Fig. 3*d–g*). Total emission count in Fig. 3*a–c* saturated the amplifier, thus accounting for the apparent constant emission rate against time. Note also that samples T23 and T24 were both multiple (2) fractures with times between the fractures of 55 ms and 40 ms respectively. The most prominent feature in Fig. 3 is the distinct decrease in the emission rate occurring simultaneously with the precursory tilt for each fracture in T23 and T24. The time durations of both the tilt and seismicity anomalies are ~ 1–2 ms. The seismicity decrease occurs during the tilt reversal before the formation of each fracture in Fig. 3.

Discussion

A scale invariant theory of failure governing the initiation and growth of faulting in rock has been proposed[2]. The scale invariant properties of failing rock provide an explanation of

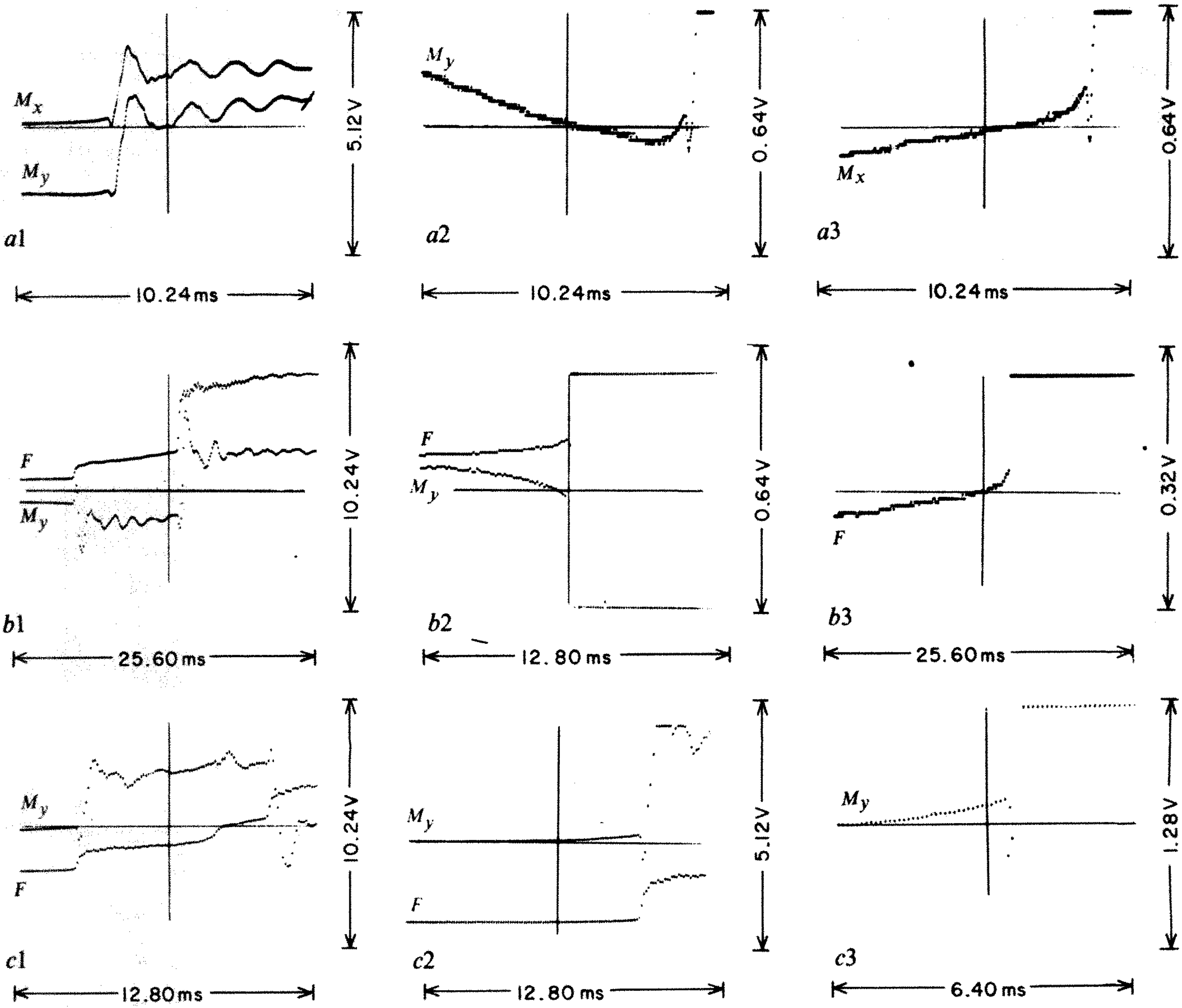
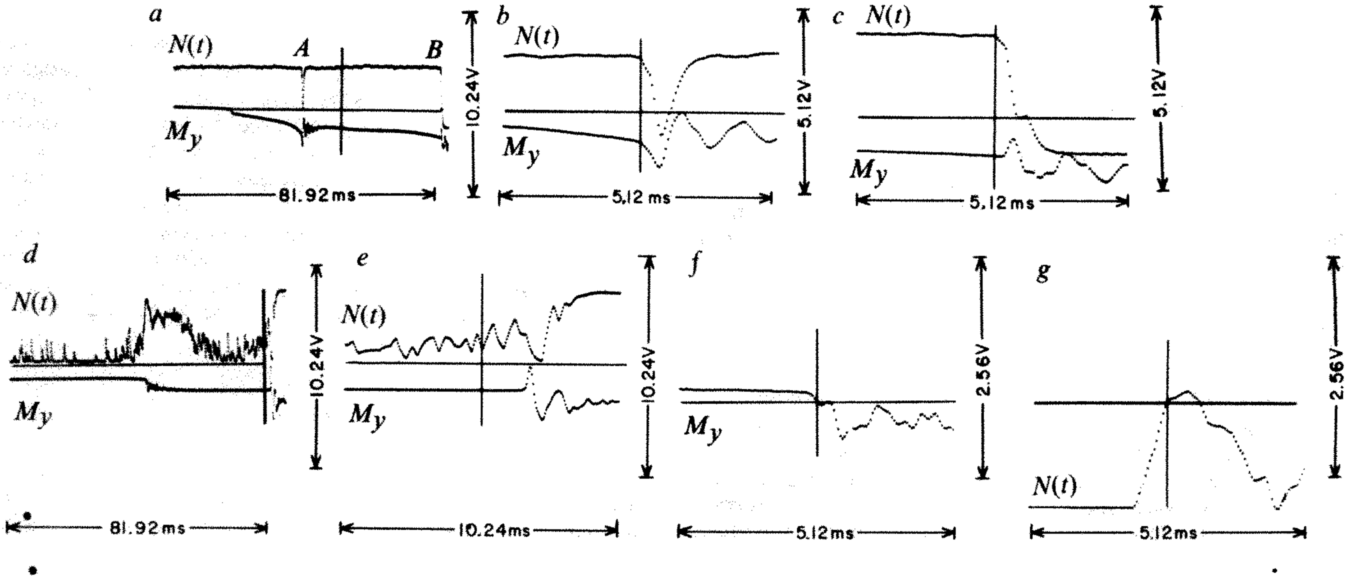


Fig. 2 Moment and load variation with time for Barre granite. *a*, BG-T14: vertical scale lengths for M_x and M_y are 8.65×10^2 N m V^{-1} and 1.03×10^3 N m V^{-1}; *b*, BG-T29: vertical scale lengths for F and M_y are 9.08×10^4 N V^{-1} and 5.86×10^2 N m V^{-1}, respectively; *c*, BG-T30: vertical scale length for F and M_y are 9.08×10^4 N V^{-1} and 5.86×10^2 N m V^{-1} respectively.

Fig. 3 Seismicity and tilt behaviour against time for Barre granite specimens T23 (*a–c*) and T24 (*d–g*). *b* and *c*, Specify tilt and seismicity for fractures *a* and *b*, respectively, of specimen T23. The conversion factor for M_y (lower trace) is 5.86×10^2 N m V^{-1}. Vertical scale $N(t)$ (upper trace) for seismicity denotes $\log_{10} n(t)$ and represents the total emission count occurring within each 200-μs time window. The conversion factor for the total count is $\log_{10} n(t) = 10\ V$, where V denotes voltage. Bandpass range is 0.3–1 MHz (*a–c*) and 1–2 MHz (*d–g*).



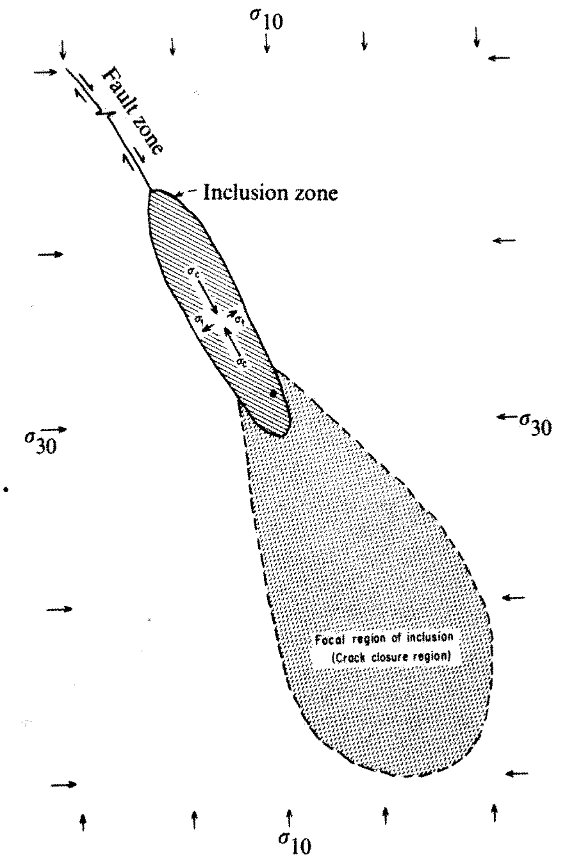


Fig. 4 Schematic illustration of fault zones, inclusion zone and focal region of the inclusion. Maximum and least principal stress applied at distances far removed from the inclusion boundary are indicated by σ_{10} and σ_{30}, respectively.

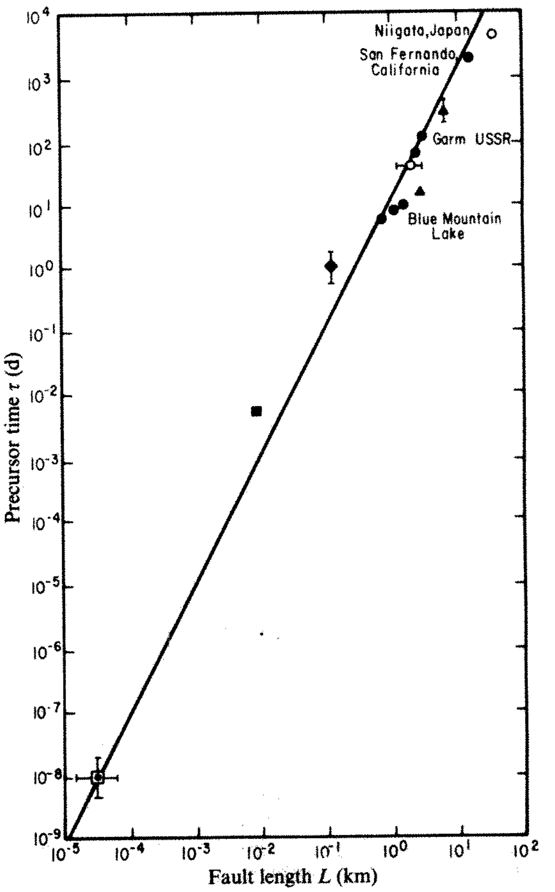


Fig. 5 Precursor time–fault length relationships for selected earthquakes, mine failures and laboratory failures. □, Laboratory seismicity and tilt measurements; ■ noise count for a coal-mine rock fall; ◆ rock burst; ● V_p/V_s anomaly; ▲, radon emission from Tashkent; ○, crustal movements.

why the precursor time–magnitude–'fault' lengths relationships hold for both mine failures and earthquakes[2]. Very briefly, the key hypothesis of this theory, termed the inclusion theory, is that the intense crack concentrations, experimentally observed to develop within localised regions in rock just before failure, can be mathematically modelled, and physically replaced by low modulus inclusions. The inclusions produce a threefold effect. First, the local principal stress axes rotate both within and outside the inclusion during the formation of the inclusion. Second, a state of tension stress is set up within and normal to the long axis of the inclusion, and third, the magnitude of the least principal stress increases in compression in the focal region of the inclusion, that is, the region into which the inclusion and its associated fault(s) will grow (Fig. 4). Thus, as the least principal stress increases, cracks (if present) in the focal region of the inclusion begin to close as the focal region begins to store strain energy, some of which will eventually be released as seismic radiation at the moment of failure. In this theory, the precursor time is defined to be the interval between the initiation of crack closure and failure, that is, the time between inclusion zone formation and the occurrence of catastrophic failure. The theory rests on the postulate that failure occurs when all cracks in the focal region of the inclusion close. At this point, the elastic contrast between the inclusion and its surroundings, the tension stress within the inclusion and the strain energy density throughout the focal region all attain their maximum possible values, and the process of catastrophic failure begins. Data in Figs 1, 2 and 3 support the hypothesis.

Anomalous tilt and seismicity behaviour before failure are predicted by the theory. For example, during the formation of the inclusion zone, tilting will be in a direction away from this zone. The tilt direction will reverse as cracks close in the focal region. Seismicity within the focal region of the inclusion will decrease in response to the decrease in the principal stress difference in the focal region of the inclusion. The theory also predicts that the time duration of these anomalies is independent of rock type and is a function only of the size of the failure. For example, a time anomaly on the order of a few milliseconds will occur before failure in laboratory size (~1–10 cm length) specimens.

Figure 5 illustrates precursor time–'fault' length for selected earthquakes[6], mine failures[1] and laboratory failures (based on the above experimental data). Fault length, assumed to be proportional to inclusion zone length for the laboratory specimens, was estimated to be in the range 1–10 cm. Note the time anomalies for the laboratory failures lie where predicted. Thus the scale-independent properties of the rock failure, at least within a precursor time interval of 10^{-8}–10^4 d or, alternatively, seven orders of magnitude in 'fault' length, seem to have experimental justification. Tests on other rock types, such as oil shale, schist, marble and hydrostone, suggest tilt precursor anomalies also of the order of a few milliseconds. Further study is, however, required to test adequately the prediction that anomaly time is independent of rock type.

Dr S. Edelman of the US Department of Commerce, Bureau of Standards provided the piezoelectric transducers.

Received November 21, 1975; accepted February 6, 1976.

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Stable nuclear activation dependent on a protein synthesised during oogenesis

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A protein synthesised during oogenesis seems to be essential for the activation, during blastulation, of the nuclear genes essential for gastrulation and organogenesis. Nuclear transplantation experiments show that this interaction between the protein and the blastula nucleus produces a heritable state of nuclear activation.

It was established by the classical experimental embryologists[1-3] that the morphogenetic pattern of early development is already present in the cytoplasm of the fertilised egg. These morphogenetic substances are produced during oogenesis, stored in the egg cytoplasm and, after fertilisation, control the normal development of the zygote into the differentiated multicellular organism. The nature of these morphogenetic substances and the manner in which they function are unknown. Genes which exert maternal effects through modifications of the egg cytoplasm are therefore of special interest, since they provide a means of approaching the problem of how the egg cytoplasm acts in controlling early embryonic development[4-8].

The o^+ substance

A simple recessive maternal effect gene (*o* for ova deficient), has been discovered in the Mexican axolotl (*Ambystoma mexicanum*). Females homozygous for *o* produce eggs which always arrest at gastrulation, regardless of whether or not the normal allele is introduced by the sperm at fertilisation. The genetic lesion occurs within the ovary and its expression is not affected by the maternal environment[9]. This suggests a gene-controlled modification of the egg cytoplasm affecting fundamental events in early morphogenesis. This modification is known to be in the nature of a cytoplasmic deficiency. The injection of nucleoplasm from a normal oocyte nucleus (germinal vesicle) or cytoplasm from a normal mature egg (after the germinal vesicle breaks down) into a mutant egg completely corrects the arrest at gastrulation[10]. The active component recovered from the oocytes of widely separated amphibian species seems to be functionally equivalent in the regulation of early embryonic development[11].

The normal allele of the *o* gene seems to direct the synthesis of a substance during oogenesis. This o^+ substance is present in the germinal vesicle by early "lampbrush" stage and its synthesis continues through most of oocyte growth. After the breakdown of the germinal vesicle the o^+ substance is found in the cytoplasm of mature eggs[11]. The active factor cannot diffuse between the blastomeres[10], and is no longer present in detectable amounts after the late blastula stage, suggesting that it has become fixed to some cell structure, rendering it unextractable, or that it has been degraded. The substance seems to be a protein(s) or to depend on protein(s) for its activity[12,13]. Preliminary results indicate that it might be a high molecular weight acidic protein (G. M. Malacinski, personnal communication).

The first differences between the mutant and normal eggs appear at mid-blastula stage. Before this, mutant and normal eggs exhibit essentially the same pattern of protein synthesis, as compared by double isotope acrylamide gel electrophoresis experiments. At the late blastula stage, however, eggs retain the pattern of protein synthesis seen during earlier stages, whereas the normal eggs show an increased relative rate of synthesis of some proteins. This failure to alter or modulate the pattern of protein synthesis is also exhibited by mechanically enucleated amphibian eggs[14,15].

Amphibian development can proceed through blastulation without a functional genome; gastrulation, however, requires the presence of a nucleus[3,16]. In *Xenopus* a spurt of new gene activity during mid-to-late blastula stages produces a population of new transcripts before gastrulation[17]. Normal axolotl eggs show transcription of various RNAs at mid-blastula stage. In contrast mutant blastulae show no incorporation of ^{3}H-uridine autoradiographically[18], but may display small amounts of incorporation by biochemical fraction analysis (R. Steele, A.J.B. and G. M. Malacinski, unpublished). These results suggest that transcription in the mutant blastula may be defective. An analysis of the pattern of RNA synthesis in both normal and mutant eggs is in progress. The data indicate that the o^+ substance is a protein(s) synthesised during oogenesis, stored in the ooplasm, and acting after fertilisation to control early developmental events. The presence of this protein(s) in the egg cytoplasm seems to be essential for the normal activation, during blastulation, of the nuclear genes required for gastrulation and organogenesis. A test of the stability of this nuclear activation is reported here.

Stable nuclear activation

A test can be obtained of the heritability of the nuclear activation by transplanting nuclei (which have been exposed to the o^+ substance) from various stages of normal blastulae into mutant eggs (which lack the o^+ substance). If the activation is not stable, then the recipient eggs, should in all cases develop like typical mutants—all arresting in gastrulation. If the activation is heritable, then the activated nuclei, but not the unactivated ones, should promote normal development of the

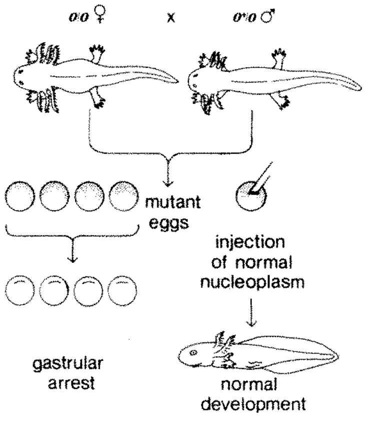

Fig. 1 A maternal effect mutation (*o*, for ova deficient) in the Mexican axolotl. Females homozygous for *o* produce eggs which always arrest at gastrulation. This gastrular arrest can be corrected by the injection of o^+ substance. This o^+ substance is produced during oogenesis under the direction of the normal allele of the *o* gene. The substance is found in the germinal vesicle and in the cytoplasm of normal mature eggs.

Table 1 Capacity of normal blastula nuclei to promote development of recipient mutant eggs

Developmental stage of the nuclear donor	Total (100%)	Number (%) of embryos surviving to each stage Complete blastula	Early-gastrula (stage 10)	Neurula	Post-neurula	Larva	Feeding larva (1 month post-hatching)
Fertile mutant eggs —no transplant	888	883 (99%)	883 (99%)	0			
Early blastula (stages 8–8¼)	234	130 (56%)	127 (54%)	0			
Mid-blastula (stage 8½)	87	41 (47%)	37 (42%)	25 (29%)	21 (24%)	18 (20%)	16 (18%)
Late mid-blastula (stage 8¾)	56	27 (48%)	24 (43%)	19 (34%)	16 (28%)	15 (27%)	13 (23%)
Late blastula (stage 9)	141	85 (60%)	69 (49%)	49 (35%)	46 (33%)	37 (25%)	28 (20%)
Late gastrula (stages 11½–12)	218	122 (56%)	75 (34%)	58 (27%)	43 (20%)	39 (18%)	25 (11%)
Control—transplants of nuclei from a normal blastula into an enucleated normal egg							
Early blastula (stage 8)	157	80 (51%)	75 (48%)	63 (40%)	54 (34%)	49 (31%)	37 (23%)
Late blastula (stage 9)	108	67 (62%)	66 (61%)	57 (53%)	48 (44%)	33 (30%)	29 (28%)
Mid-gastrula (stages 10½–11½)	98	56 (57%)	50 (51%)	36 (37%)	24 (24%)	22 (22%)	17 (17%)

A preliminary report of some of these results has been published in ref. 19. The nuclear transplants were done by an alteration (A. J. Brothers, unpublished) of the previously described techniques[20]. The staging series used is a modification of the stage series described by Harrison[21] and Carroll[18]. The modifications used in this paper are based on the developmental stage, measured by the time after first cleavage, at 18 °C. Stage 8=15–18 h. Stage 8¼=18–20 h. Stage 8½=20–22 h. Stage 8¾=22–24 h. Stage 9=24–29 h. The genetic markers used for the nuclear transplants were those governing pigmentation (*d*), (*m*) and in some instances recessive mutations affecting early development (*f*), (*g*) and a cell autonomous lethal (*ut*) (refs 4, 13).

recipient enucleated mutant eggs. Transplantation of nuclei taken from normal early blastulae shows that those nuclei cannot improve the development of the recipient mutant eggs. Nuclei taken from normal mid-to-late blastulae are, however, capable of promoting normal development of the mutant eggs. (A preliminary report on the stability of late blastulae nuclei to support the development of recipient mutant eggs has been published[19].) More complete results indicate that the stable alteration of the ability of the nucleus to support normal development is established at mid-blastulation. (Table 1).

Some of the animals derived from these transplants have been raised to sexually mature adults (Fig. 2). The spawnings from those animals developed normally and the genetic markers of the donor nuclei were expressed. A small amount of cytoplasm necessarily accompanies the transplanted nucleus. The contribution of this amount of cytoplasm to the correction is probably negligible, since at least 2% of the whole cytoplasm from a normal mature egg must be injected into a mutant egg in order to obtain correction[10]. Also, the active factor is no longer extractable from blastula stages, suggesting that it has become degraded or fixed to some cellular structure[12]. Most important however, is the observation that normal nuclei from early blastulae do not show any capacity to alter the mutant phenotype when transplanted into mutant eggs, whereas normal late blastulae nuclei can support normal development.

Crude nuclear homogenates[22] were made from normal late blastulae and aliquots were injected into fertile mutant and normal eggs. No correction of the phenotype of the mutant eggs were observed.

These observations suggest that the correction in the nuclear transplants is not fortuitously due to the accompanying cytoplasm nor is it due to some diffusible substance concentrated within the blastula nucleus. Therefore, the ability of normal late blastula nuclei to support development of the recipient mutant eggs must be a property of the intact nucleus itself.

Heritability of nuclear activation

A definitive test of the heritability of the activation was provided by serial clonal nuclear transplants of activated nuclei, using enucleated mutant eggs for recipients at each transplant generation (Fig. 3). The nuclei were transplanted into enucleated mutant eggs, and those transplants were allowed to develop to early-blastulae (stage 8). At this point those nuclei had undergone at least nine mitotic divisions[22] in the cytoplasmically deficient mutant eggs. Previous experiments had shown that nuclei from stage 8 blastulae had not yet exhibited activation, as measured by the capacity to promote normal development when transplanted into mutant eggs. Therefore, the serial clonal transplants would provide a test of whether or not a nucleus, once it has passed through the activation period, retains the capacity to support normal development through gastrulation and neurulation.

Some of the transplants in each series were taken at stage 8 to provide nuclear donors for the serial clonal transplants and a small portion of each nuclear donor blastula was also used for the determination of the autoradiographic pattern of ^{3}H-uridine incorporation. The nuclei were again transplanted into enucleated mutant eggs, creating serial transplant clones. Members of each of those clones developed into normal larvae and showed the expression of the genetic markers of the original donor nuclei (Table 2). These experiments demonstrate that the nuclear activation (the capacity to support the normal development of early morphogenetic events) is a stable and heritable condition for more than 30 mitotic generations.

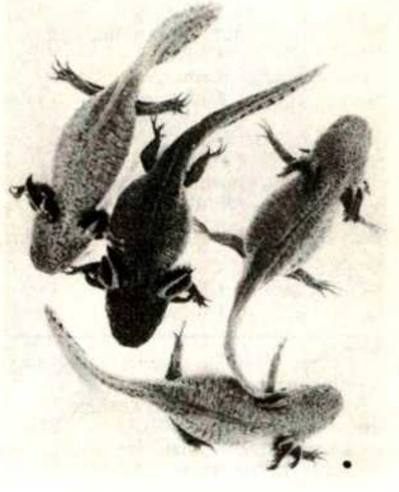

Fig. 2 Adult axolotls derived from nuclear transplants of stage 9 normal blastula nuclei into enucleated mutant eggs.

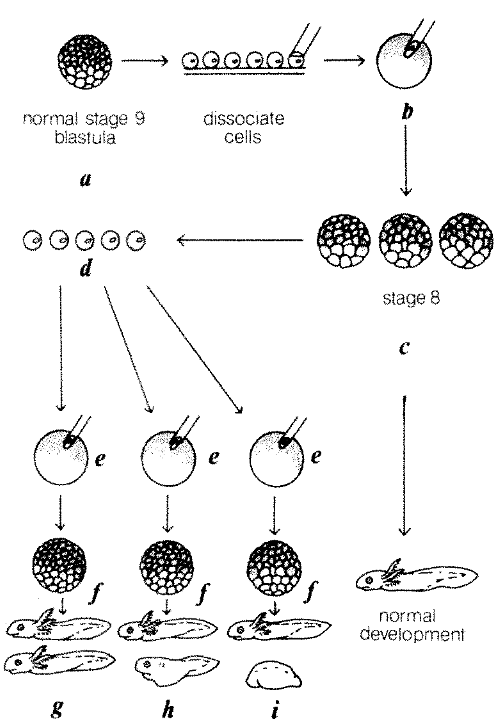

Fig. 3 Serial clonal nuclear transplants of activated nuclei using enucleated mutant eggs for recipients at each transplant generation. *a*, The nuclear donor is a normal stage 9, late blastula which shows intense ^{3}H-uridine incorporation in autoradiographs; *b*, the dissociated cells are used for nuclear transplants into an enucleated mutant egg; *c*, the nuclear transplants are allowed to develop to stage 8, early blastula stage. A few are sacrificed to serve as nuclear donors for the serial clonal transplants, and a portion of each nuclear donor blastula is taken for autoradiographic analysis. There is no detectable ^{3}H-uridine incorporation. *d*, The stage 8 nuclear donor blastula is dissociated in Ca^{2+}- and Mg^{2+}-free Steinberg's medium plus 0.005 M Na citrate, *p*H = 7.4 (ref. 48); *e*, serial clonal nuclear transplants are done, again using enucleated mutant recipient eggs; *f*, the serial clonal transplants are allowed to develop to stage 9, late blastula stage, where a few blastula from each serial clone are used for autoradiographic analysis. They show intense ^{3}H-uridine incorporation.

The capacity to express the nuclear genes necessary for gastrulation and organogenesis is stably altered, apparently independently of continued RNA synthesis (as measured by autoradiographic methods).

Normal early blastulae (stage 8) exhibit little or no nuclear incorporation of ^{3}H-uridine, but normal late blastulae (stage 9) show intense nuclear labelling. In contrast, mutant blastulae never show incorporation of ^{3}H-uridine autoradiographically[18]. Analysis of serial clonal transplants of normal late blastula nuclei into recipient mutant or normal eggs shows that the autoradiographic incorporation pattern of ^{3}H-uridine is the same as the pattern exhibited by control normal eggs.

In *Xenopus* rRNA, synthesis is not detectable until late cleavage or gastrula stages. Gurdon and Brown demonstrated that somatic cell nuclei which were synthesising rRNA, when transplanted into enucleated recipient eggs, did not continue synthesising rRNA in detectable amounts, but exhibited a pattern of rRNA synthesis typical of zygote nuclei[24]. Cytoplasmic influence on the pattern of gene expression has also been described for somatic cell hybrids and heterokaryons[25,26].

o^+ Substance interaction with nucleus

The ability of an embryonic nucleus to support development up through neural stages is dependent on an interaction with the o^+ substance. Injection of plasma containing o^+ substance into fertile mutant eggs enables them to develop normally, demonstrating that the nuclei of mutant eggs can respond to the o^+ substance[10–13].

Transplantation of nuclei from mutant blastulae into normal enucleated recipient eggs tested the question of whether or not nuclei retain the capacity to interact with the o^+ substance throughout early development. As nuclei of mutant eggs undergo cleavage they are not exposed to the o^+ substance, since those eggs lack it. The transplantation of such nuclei into normal eggs, which contain the active factor, represents the first contact that those nuclei have with the o^+ substance. The ability of those nuclei to support normal development would provide evidence that they responded to the o^+ substance in the cytoplasm of the recipient normal eggs.

The experimental results (Table 3) demonstrate that late-cleavage (stage 7) and early-blastulae (stage 8½) mutant nuclei

Table 2 Serial clonal transplants of activated nuclei into mutant eggs

		No. of embryos surviving to each stage					
	Total	Complete blastula	Gastrula	Neurula	Post-neurula	Larva	Feeding larva
Series *A*							
Late blastula, normal stage 9 nucleus ($o^+/o^+, fg/fg^+$) put into a mutant egg	18	9*	6	6	6	5	4
Clone 1*a*	13	3	3	3	3	3	3
Clone 2*a*	18	5	5	5	3	1	0
Clone 3*a*	19	12	11	10	10	5	4
Series *B*							
Late blastula, normal stage 9 nucleus ($o^+/o^+, d/d, ut/ut^+$) put into a mutant egg	43	21†	15	13	10	7	6
Clone 1*b*	15	9‡	7	5	5	4	4
Clone 2*b*	17	10‡	6	4	2	1	1
Clone 3*b*	18	13‡	9	7	5	5	4
Clone 4*b*	13	9‡	6	5	2	2	1

*Three blastulae taken at stage 8 to serve as donors for nuclei for the clonal transplants (1*a*–3*a*) and for autoradiographic analysis of ^{3}H-uridine incorporation.

†Four blastulae taken at stage 8 to serve as donors for nuclei for the clonal transplants (1*b*–4*b*) and for autoradiographic analysis of ^{3}H-uridine incorporation.

‡A few stage 9 blastulae taken from each clone to check for autoradiographic incorporation of ^{3}H-uridine.

A preliminary report of the results obtained from series *A* serial clonal transplants has been published[19].

Table 3 The capacity of the embryonic nucleus to interact with the o^+ substance in the egg cytoplasm

Developmental stage of the nuclear donor	Total (100%)	No. (%) of embryos surviving to each stage						
			Cleavage		Blastula			
		Uncleaved	Abnormal	Normal	Partial	Complete	Neurula	Larva
Transplantation of a mutant blastula nucleus into an enucleated normal egg								
Late-cleavage (stage 7)	39	3 (8%)	11 (28%)	25 (64%)	8 (20%)	23 (59%)	18 (46%)	12 (31%)
Early-blastula (stages 8–8¼)	53	4 (8%)	4 (8%)	45 (84%)	6 (11%)	39 (73%)	29 (54%)	19 (36%)
Late mid-blastula (stage 8¾)	38	2 (5%)	6 (16%)	30 (79%)	18 (47%)	12 (31%)	0	—
Late-blastula (stage 9)	67	44 (66%)	23 (34%)	0	7 (10%)	0	0	—
Control—transplantation of a normal blastula nucleus into an enucleated normal egg								
Late-blastula (stage 9)	36	6 (17%)	5 (14%)	25 (69%)	4 (11%)	25 (69%)	19 (53%)	12 (33%)

have the capacity to respond to the o^+ substance and that this response acts to alter the expression of the mutant phenotype. After advanced mid-blastula (by stage 8¾) this capacity is lost; nuclei at this stage of development and older are no longer capable of supporting normal development if they have not been previously exposed to the o^+ substance. Mutant late blastulae (stage 9) show few or no mitoses, or incorporation of ^{3}H-thymidine[18]. Transplantation of those nuclei into normal eggs indicates that they are incapable of supporting normal cleavage. This suggests that late-blastula nuclei which have not been activated by an exposure to the o^+ substance are injured and are unable to function properly, even when exposed to normal cytoplasm.

Mode of action of o^+ substance

Cytoplasmic control of gene expression has been amply documented[24–28]. The migration of proteins between the cytoplasm and the nucleus has been reported for amoebae[29], insects[30–31] and amphibians[32–34], as well as for many heterokaryons[35]. Recent experiments have demonstrated the differential accumulation of certain classes of protein in nuclei[31,36]. The association of proteins with DNA or the chromosomes, and the subsequent modification of DNA or chromosome behaviour has been described for various systems[41–43].

The o^+ substance seems to be a protein, which is apparently needed to establish the stable activation of the capacity to express the genes required to support normal development through gastrulation and organogenesis. It is tempting to speculate that the o^+ substance might be a regulatory protein, such as Davidson and Britten discussed in their model for eukaryotic gene regulation[44]. The experimental results reported in this paper show that the nuclear activation is dependent on the presence of the o^+ substance within the cytoplasm of the fertilised egg. But these experiments do not show whether there is a direct interaction of the o^+ substance and the embryonic genome. Experiments to settle this issue are now in progress. In addition, the pattern of RNA synthesis in both normal and mutant eggs is currently under examination. It is expected that this approach will provide some insight into the possible relationship between the nuclear activation and gene expression.

Sonneborn's elegant experiments on the inheritance of mating type in *Paramecium* provide another demonstration of a cytoplasmically controlled stable nuclear determination. The sensitive period for this nuclear determination is restricted to one point in the nuclear "life cycle". During this period a cytoplasmic factor acts to produce a genetic alteration in the macronucleus (T. M. Sonneborn, personal communication). This is a stable alteration and is heritable through all subsequent clonal generations, even if the nucleus is later exposed to cytoplasm from the opposite mating type[45].

Cellular determination is endowed with a high degree of stability[25,46]. Once it is established, the capacity to express a specific state of determination can be inherited over many cellular generations, even though the differentiated functions are not expressed[46,47]. The pattern of gene expression can be controlled and modified by cytoplasmic or extracellular factors[25,27,41–43].

In the axolotl the o^+ substance is produced during oogenesis, and the presence of this protein(s) in the egg cytoplasm is essential for the establishment of a stable nuclear activation. The sensitive period for this activation is restricted to one point during embryonic development. Apparently this alteration of the capacity to support development is the result of the activation of the nuclear genes required for gastrulation and neurulation. The isolation and characterisation of the o^+ substance provides an opportunity to examine the manner in which a morphogenetic substance functions.

I thank R. Briggs, R. R. Humphrey, G. M. Malacinski and T. M. Sonneborn for discussions and comments. This work was supported by the National Science Foundation and the National Institutes of Health.

Received October 23, 1975; accepted January 19, 1976.

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Injected nuclei in frog oocytes provide a living cell system for the study of transcriptional control

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Human HeLa nuclei injected into Xenopus *oocytes synthesise RNA continuously for up to 1 month, and some of this RNA is translated into HeLa proteins. Nuclear proteins extracted from other cells enter the injected nuclei. This living cell system can be used for the study of transcriptional controls.*

THE identification of chromosomal molecules that regulate gene transcription requires experimental systems in which the function of purified macromolecules can be tested efficiently. Cell-free systems which engage in transcription from isolated nuclei or chromatin are usually characterised by low efficiency (rate of transcription *in vitro* compared with that of the same nuclei *in vivo*), few new initiations (compared with *in vivo*), and sometimes inaccurate reading[1,2] (choice of DNA strand and initiation or termination points). Even in the best systems involving isolated nuclei (for example, ref. 3), transcription has almost ceased within 2 h, and much of the labelled RNA obtained results from completion of chains initiated *in vivo*, rather than from new initiations *in vitro*. We have therefore explored the usefulness of living frog oocytes, which can be injected with nuclei and/or purified macromolecules as a system for investigating transcriptional controls. Earlier work has shown that *Xenopus* blastula nuclei continue to synthesise RNA for 3 d after injection into oocytes[4]. At the level of protein synthesis, reinitiation takes place on mRNA injected into oocytes for up to 2 weeks, as a result of which each molecule of mRNA may be translated up to 100,000 times[5]. We conclude from our results that human cultured cell nuclei injected into oocytes are transcriptionally active for up to 1 month, that some of their genes are expressed as newly synthesised proteins, and that they take up injected and endogenous proteins from the surrounding oocyte cytoplasm.

Persistence of injected nuclei in oocytes

The fate of ^{3}H-thymidine (TdR)-labelled HeLa nuclei in *Xenopus* oocytes has been followed by autoradiography after fixing and sectioning. Oocytes of *Xenopus laevis* were prepared, injected and cultured as described previously[4,6,7], at 19 or 25 °C. In these conditions, oocytes appear morphologically normal for up to 1 month and injected nuclei remain intact. During the first few days after injection, nuclei undergo a substantial enlargement of eight times or more in volume, and their chromatin becomes dispersed (Figs 1*b* and 2*b*). Nuclei injected into the cytoplasm of an oocyte usually come to lie close to the oocyte nucleus (germinal vesicle) (Fig. 1*b*). With practice, it is possible to inject nuclei into the germinal vesicle (oocyte's nucleus), which is obscured in living oocytes by pigment and yolk; in most such experiments nuclei are deposited in an intact germinal vesicle or in the region where it breaks down. In these conditions, the injected nuclei can enlarge enormously (up to 100 times in volume), and may occupy the whole of the space inside the germinal vesicle whose chromosomes are displaced (Fig. 1*a*).

We have found that several different methods can be used successfully to prepare nuclei for injection. In all cases the nuclei injected are surrounded by cytoplasm; however, for convenience, they are referred to here as nuclei. The method used for many of the experiments reported here is to suck cells into a small pipette so that the cell is ruptured, but cytoplasm not removed from around the nucleus—the method used to transplant nuclei to eggs[6]. It is essential however, not to prepare nuclei with the aid of detergents such as Triton X-100 or Nonidet P40; nuclei prepared in this way always die a few days after injection into oocytes. We usually inject about 200 HeLa nuclei into each oocyte.

Injected nuclei synthesise RNA for many days

Oocytes containing injected nuclei have been labelled by incubation in medium containing ^{3}H-guanosine (^{3}H-G) and ^{3}H-uridine (^{3}H-U) or by a second injection of ^{3}H-guanosine triphosphate (^{3}H-GTP). After the desired time, labelled oocytes are fixed, sectioned and autoradiographed. In all, oocytes tested for RNA synthesis (more than 200), nuclei injected into the cytoplasm or germinal vesicle were heavily labelled (Fig. 2*d*), whether the labelling period was as short as 30 min (after ^{3}H-GTP injection), or as long as 36 h. Furthermore, nuclei seemed to synthesise RNA continuously throughout incubation. Even when oocytes containing HeLa nuclei were incubated in non-radioactive medium for 28 d at 19 °C and then in medium with ^{3}H-U and ^{3}H-G for a further 24 h, the injected nuclei were labelled strongly.

The amount of labelling over injected nuclei can be compared with that of the oocyte's germinal vesicle which serves as a convenient internal standard. For all labelling periods (0.5–24 h

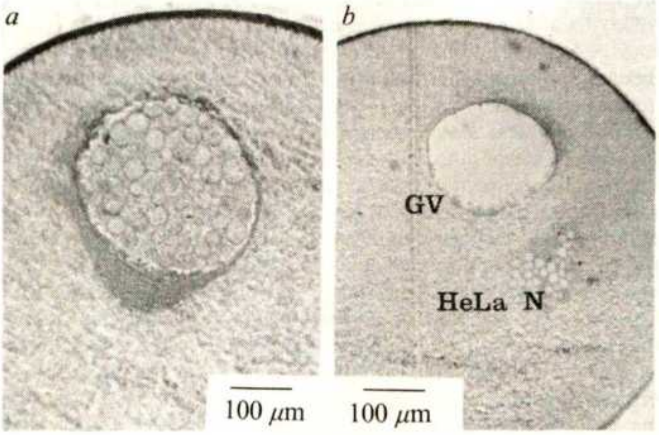

Fig. 1 *Xenopus* oocytes containing injected HeLa cell nuclei. GV, Germinal vesicle or nucleus of the oocyte; HeLa N, injected HeLa nuclei. *a*, GV filled with numerous enlarged HeLa nuclei; *b*, HeLa nuclei in the cytoplasm of an oocyte with an intact GV. Some of the nuclei in (*b*) are shown at a higher magnification in Fig. 2*b*. Oocytes were fixed 3 d after injection of nuclei. Cells used to provide nuclei were suspended in 0.25 M sucrose and 2 mM $MgCl_2$. HeLa cells were grown in plastic bottles by standard procedures, and checked for PPLO periodically.

at 1–28 d after injection), HeLa nuclei were more heavily labelled than the nucleoplasm of the germinal vesicle, but less so than the nucleoli in the germinal vesicle (Fig. 2*c* and *d*). It is also observed that greatly swollen nuclei (those injected 4 or more days previously) are about as heavily labelled, per area of section, as small, unswollen nuclei (those injected within the previous day). Nuclei therefore seem to increase their rate of RNA synthesis in proportion to their enlargement during incubation in oocytes. This effect is in striking contrast to the dilution of label observed during the enlargement of nuclei containing prelabelled DNA. Very rough estimates, based on the known rate of RNA synthesis by amphibian oocytes[8,9], indicate that the HeLa nuclei synthesise RNA at a rate which is at least the same in oocytes at 19 °C as in HeLa cells at 37 °C (refs 10 and 11). Sometimes a few of the injected nuclei fail to enlarge; after a few days they become pycnotic and cease to synthesise RNA.

The incorporation of ^{3}H-U+^{3}H-G or of ^{3}H-GTP by injected nuclei as just described is assumed to represent RNA synthesis, for the following reasons. The labelling of injected nuclei and of the oocyte germinal vesicle with its nucleoli is almost entirely eliminated by treatment with RNase before autoradiography. The germinal vesicles and especially nucleoli of *Xenopus* oocytes are known to be very active in RNA synthesis and inactive in DNA synthesis[12–14], and their pattern of labelling in these experiments was as previously observed. The possibility that label over injected nuclei represents the accumulation in them of RNA synthesised in, and transported from, the germinal vesicle is unlikely for two reasons. First, injected nuclei show the same level of labelling relative to the germinal vesicle after very short (30 min) or very long (36 h) labelling periods. Second, the injection of HeLa nuclei containing labelled RNA into unlabelled oocytes does not result in transfer of this RNA to the germinal vesicle, a fact which argues against a significant exchange of labelled RNA between nuclei in the same oocyte.

The results described so far indicate that HeLa nuclei remain transcriptionally active for at least 1 month. Since they seem to synthesise RNA at a substantial rate, we assume that extensive reinitiation of transcription takes place during this period.

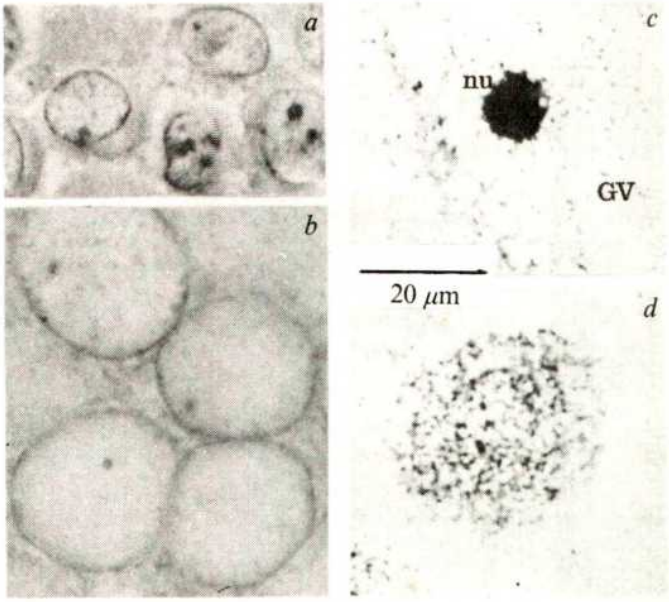

Fig. 2 HeLa nuclei in *Xenopus* oocyte cytoplasm. *a*, HeLa nuclei 5 min after injection; *b*, swollen HeLa nuclei 3 d after injection; *c*, autoradiograph of the germinal vesicle (GV) and a nucleolus (nu) of an oocyte; *d*, autoradiograph of a HeLa nucleus in oocyte cytoplasm. Both (*c*) and (*d*) show the same oocyte which, 3 d after injection of HeLa nuclei, was incubated in ^{3}H-U and ^{3}H-G for 24 h. In (*d*), the HeLa nucleus is more heavily labelled than the nucleoplasm of the oocyte but less heavily than the nucleolus (obscured by grains).

Some HeLa genes are expressed in frog oocytes

Although HeLa nuclei actively synthesise RNA in frog oocytes, the results described so far do not show that this RNA includes mRNAs which code for any identifiable gene products. We have used two-dimensional isoelectric focusing—sodium dodecyl sulphate gel electrophoresis, the high resolution method of O'Farrell[15]— to detect HeLa-coded proteins in oocytes containing injected nuclei. Oocytes injected with HeLa nuclei were incubated in culture medium[6,16] containing mixed ^{14}C-amino acids. Proteins were extracted from the labelled oocytes, electrophoresed in two dimensions, and the resulting slab gels were dried and fluorographed[17,18].

Xenopus oocytes, labelled with a ^{14}C-amino acid mixture, give a highly reproducible distribution of proteins (Fig. 3*a*). When oocytes with and without HeLa nuclei were labelled for 6 h immediately after injection, and their proteins were compared, no difference was observed. But when oocytes with and without HeLa nuclei were incubated for 3 d at 25 °C, labelled for 6 h and analysed by the same procedure, some extra proteins were seen in the nucleus-injected oocytes. One of these proteins, named 66/6.04 (because it has a molecular weight of 66,000, and an isoelectric point of 6.04), is strongly labelled (compare Fig. 3*b* and *c*). Proteins synthesised by HeLa cells include one with these properties, but mouse myeloma cells, and oocytes injected with myeloma nuclei (labelled after 3 d), do not (Fig. 3*e*). To characterise further this protein, region 66/6.04 was cut out from a two-dimensional gel of proteins synthesised by intact HeLa cells and from another gel of proteins from oocytes previously injected with HeLa nuclei. Material eluted from this region was treated with cyanogen bromide, and the resulting peptides were analysed by isoelectric focusing. The peptides obtained from oocytes with HeLa nuclei and from intact HeLa cells are very well matched (Fig. 4); no detectable radioactivity was obtained from the labelled material extracted from region 66/6.04 of slab gels of uninjected oocytes. A labelled spot in position 66/6.04 has appeared in all eight independent two-dimensional analyses so far made of proteins from oocytes injected with HeLa nuclei 3 or more days previously, and in none of seven analyses of oocytes which did not contain HeLa nuclei. The spot appears whether nuclei are deposited in the cytoplasm or germinal vesicle of oocytes. We conclude that at least one HeLa protein is synthesised in oocytes injected with HeLa nuclei.

The HeLa nuclei injected into oocytes are, in effect, ruptured whole cells, and contain much of the original cells' cytoplasm. Protein 66/6.04 might therefore have been synthesised from cytoplasmic mRNA injected with the nuclei, but the following lines of evidence, although necessarily indirect, argue against this possibility and indicate that it is due to new transcription.

The new spot does not appear until about 3 d after injection, but any injected mRNA would be expected, from past experience[5,7,19], to be at least as active immediately after injection as at 3 d. This suggests that insufficient 66/6.04 message is carried over with the nuclei to be detected by two-dimensional electrophoresis during the first day after injection.

It could be argued that the observed time course might also arise if the injected nuclei were to contain mRNP particles or RNA precursors that would be translated only after processing inside the oocyte. To explore this possibility, we tested directly the amount of message carried over with our nuclear preparations. Oocytes were injected with RNA isolated from 4,000 HeLa cells prepared as for nuclear injection, that is, with 20 times more RNA than was carried in with the number of nuclei injected to give a clearly visible spot in position 66/6.04. The recovery of HeLa RNA was monitored by adding a known amount of globin mRNA to the HeLa cell extract, and obtaining a 60% recovery of its activity by translation[7,19] in the injected oocytes. The RNA-injected oocytes showed no detectable synthesis of protein 66/6.04 in the course of 3 d, even though they had received, after allowing for recovery,

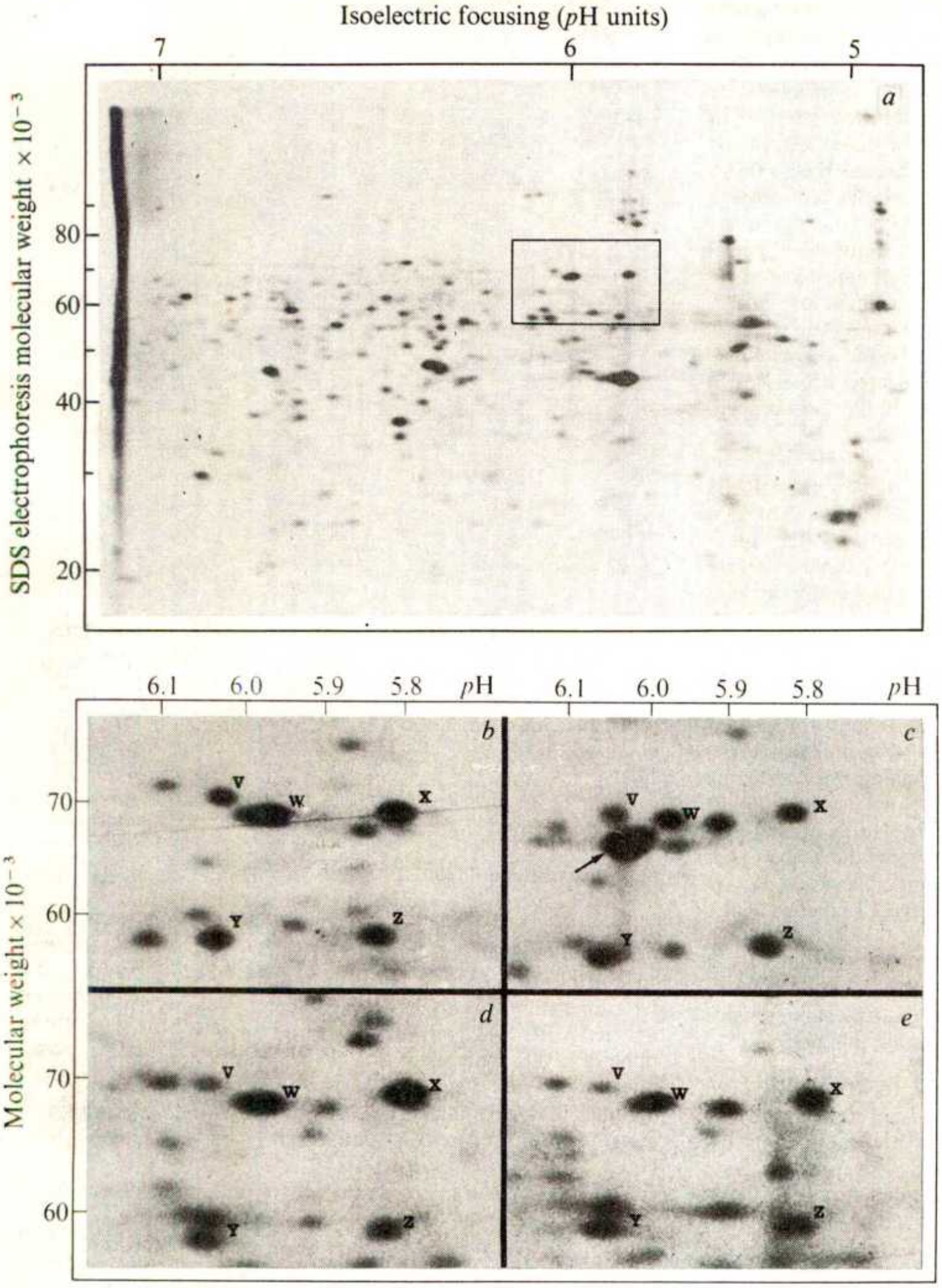
Isoelectric focusing (pH units)
7
6
5
a
SDS electrophoresis molecular weight $\times 10^{-3}$
80
60
40
20
6.1
6.0
5.9
5.8
pH
b
c
d
e
V
W
X
Y
Z
Molecular weight $\times 10^{-3}$
70
60

Fig. 3 Fluorographs of two-dimensional gels of *Xenopus laevis* oocyte proteins. Oocytes were labelled for 6 h in 2 μl per oocyte of medium containing 250 μCi per ml of L-(^{14}C-U) amino acid mixture (57 mCi per matom), 3 d after injection of nuclei (25 °C). The oocytes were homogenised in 10 mM Tris–HCl, *p*H 7.4, 5 mM MgCl$_2$, ribonuclease A 25 μg ml^{-1} and deoxyribonuclease I 25 μg ml^{-1}. After standing for 10 min on ice, the samples were made 50 mM (*a–c*), or 100 mM (*d* and *e*) in NaCl and the yolk platelets were removed by centrifugation at 1,000*g* for 5 min. The isoelectric focusing dimension was performed as described before[15] and electrophoresed for 18 h at 400 V. The second dimension was run in 15% polyacrylamide gels as described by Knowland[37]. Some of the oocyte proteins are lettered from V to Z to help comparison. In addition to the proteins induced by the injected nuclei, the oocyte background shows minor variations due to differences in the extraction procedures (for example, the double spot at Y in *d* and *e*). *a*, Oocytes injected with saline. The rectangle indicates the area magnified in fluorographs *b* to *e*. *b*, Uninjected oocytes; *c*, oocytes injected with HeLa nuclei. The arrow indicates protein 66/6.04. *d*, Oocytes injected with HeLa nuclei and α-amanitin; *e*, oocytes injected with nuclei of a mouse myeloma cell line.

about 12 times more HeLa RNA than would be carried in with injected nuclei. In addition to this experiment, the α-amanitin experiment (below) and the fact that HeLa nuclei containing adenovirus genomes at the height of their transcriptional activity do not stimulate the synthesis of adenovirus proteins in oocytes (below), also argue strongly against the processing possibilty.

If protein 66/6.04 is coded by RNA synthesised after the nuclei are injected into the oocyte, its synthesis might be expected to be sensitive to α-amanitin, an inhibitor of eukaryotic RNA polymerases[20]. Oocytes were injected with HeLa nuclei, with or without α-amanitin, cultured for 3 d, and then labelled with ^{14}C-amino acids. The synthesis of protein 66/6.04 was eliminated in the α-amanitin-treated oocytes (Fig. 3*d*) but not in the controls. Enough α-amanitin was injected to give an intracellular concentration of 10 μg ml^{-1}, found previously to inhibit the synthesis of RNAs other than 28S, 18S and 4+5S in *Xenopus* oocytes[21–23]. Several controls were performed in the same experiment. (1) Oocytes treated with α-amanitin as above showed a greatly reduced RNA synthesis over the germinal vesicle and injected nuclei, as judged by autoradiography. (2) The injected nuclei in α-amanitin-treated oocytes were morphologically normal. (3) To prove that the α-amanitin did not inhibit the translation of mRNA, purified rabbit globin mRNA was injected into some of the oocytes containing HeLa nuclei at the same time as α-amanitin, and found by one-dimensional gel anaylsis to be translated as efficiently as in other similar oocytes not injected with α-amanitin.

We conclude that protein 66/6.04 is synthesised in oocytes containing HeLa nuclei, by a coupled transcription–translation process. It seems that insufficient of the mRNA for this protein is injected with nuclei to give a labelled spot in position 66/6.04; this protein appears only when more mRNA has been synthesised by HeLa nuclei in oocytes in the course of about 3 d.

Gene expression by HeLa nuclei in oocytes is selective

Are all genes active in HeLa cells also expressed in oocytes containing HeLa nuclei? Several of the major proteins synthesised by HeLa cells are not distinguishable by two-dimensional analysis from major proteins synthesised by *Xenopus* oocytes, and the activity of these genes cannot therefore be determined in these experiments. At least sixteen major proteins are distinguishable, however. Of these, two in addition to 66/6.04 are synthesised in oocytes 3 d after injection of HeLa nuclei, but we have not detected the synthesis of the other 13. Since most kinds of vertebrate mRNA are translated efficiently after injection into *Xenopus* oocytes (for example, refs 6, 24 and 25), we presume that many genes active in intact HeLa cells become inactive in oocytes, at the transcriptional or post-transcriptional (but pre-translational) levels.

To investigate the possibility that genes in injected nuclei may be transcribed but not translated, we have injected oocytes with HeLa nuclei prepared 6 or 12 h after infection with human adenovirus type 5 at a high multiplicity. RNA synthesised by these oocytes was labelled 3 d after nuclear injection, and hybridised to purified adenovirus 5 DNA in conditions of DNA excess. Over 10^6 c.p.m. of RNA was extracted from each sample of oocytes and about 50,000 c.p.m. was used for each hybridisation reaction. The same background level of hybridisation was observed with ^{3}H-RNA from oocytes containing infected nuclei as with ^{3}H-RNA from controls. Effective operation of the hybridisation methodology and recovery of RNA was demonstrated by extracting RNA from oocytes previously injected with ^{3}H-RNA from 18-h adenovirus-infected cells. A very low level of adenovirus transcripts would not have been detected by the procedures used. This result, however agrees with that of protein analyses. Oocytes containing 6-h or 12-h adenovirus-infected HeLa nuclei synthesise protein 66/6.04, but do not synthesise two major adenovirus proteins (hexon and penton) which would have appeared in two-dimensional gel positions 115/6.10 and 70/7.00. Nor are the infected cell-specific polypeptides which would have been seen in positions 95/6.10, 78/6.00 and 65/6.25 synthesised by these oocytes. Apparently the adenovirus genome becomes transcriptionally inactive soon after infected nuclei are injected into oocytes, even though transcription by some HeLa genes in the same nuclei persists.

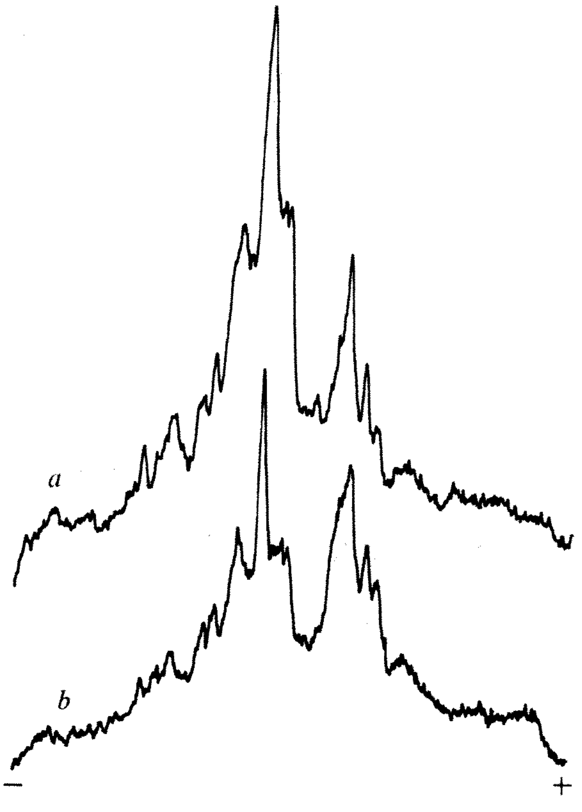

Fig. 4 Isoelectric focusing of cyanogen bromide peptides of protein 66/6.04. Total ^{14}C-proteins were electrophoresed in two-dimensional gels. After autoradiography the 66/6.04 region was cut out, homogenised and suspended in 70% formic acid containing cyanogen bromide (200 μg ml^{-1}). After shaking at 20 °C for 12 h, the gel particles were centrifuged; the supernatant was lyophilised twice, dissolved in pyridine–acetate buffer, *p*H 6.5, incubated at 60 °C overnight (to open the homoserine-lactone ring), and dried again. The samples were analysed by isoelectric focusing in a 4% polyacrylamide slab gel containing 2% ampholines (*p*H range 3.5–10) and 9 M urea. After fixing in 15% trichloroacetic acid, the gel was fluorographed and the film scanned in a Joyce-Loebl microdensitometer. *a*, Peptides from protein 66/6.04 of oocytes injected with HeLa nuclei; *b*, peptides from protein 66/6.04 of HeLa cells.

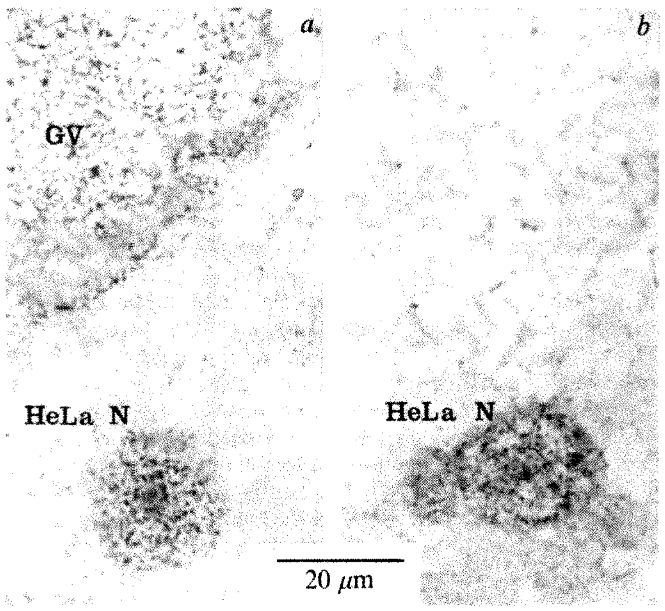

Fig. 5 *a*, Autoradiograph of an oocyte containing a HeLa nucleus. The oocyte was injected with HeLa nuclei; 5 d later it was injected with ^{125}I-labelled calf thymus histones (less H1) and incubated for a further 16 h before fixation. The HeLa nucleus (HeLa N) is about as heavily labelled as an equivalent area of the oocyte's nucleus (GV), and much more strongly labelled than the surrounding cytoplasm. *b*, Autoradiograph of a HeLa nucleus (and the edge of another one) in an oocyte; the oocyte was incubated in ^{3}H-leucine for 2 h and in unlabelled medium for a further 24 h, 4 d after it had been injected with HeLa nuclei. The nuclei are labelled much more strongly than the surrounding cytoplasm.

Uptake of proteins by injected HeLa nuclei in oocytes

If nuclei injected into oocytes are to serve as a test system for the function of macromolecules, it is essential to know that these molecules can gain access to the nuclear genes being studied. The following experiments show that some endogenous as well as introduced proteins rapidly enter HeLa nuclei from oocyte cytoplasm. Oocytes, injected with nuclei 4 d previously, were incubated in ^{3}H-leucine for 2 h, and then in excess unlabelled medium for 24 h. Autoradiography showed that the injected nuclei were strongly labelled well above the cytoplasmic background level (Fig. 5*b*). Since nuclei do not synthesise protein, they must have accumulated proteins synthesised in the cytoplasm; the fact that these proteins remained in the nuclei during a 24-h chase suggests that they may become associated with some intranuclear structures—possibly chromosomes. In another experiment, purified calf thymus histones labelled with ^{125}I (ref. 26) were injected into oocytes containing enlarged HeLa nuclei. The oocytes were fixed 24 h later, sectioned and autoradiographed. Both the injected nuclei and germinal vesicle were strongly labelled (Fig. 5*a*). The results of these experiments are supported by previous work: egg cytoplasmic proteins accumulate in endogenous or injected nuclei[27-29], whether or not puromycin is used to suppress protein synthesis, and ^{125}I-histones accumulate in oocyte germinal vesicles[30,31]. Bonner[32] has shown that non-histone proteins from an oocyte germinal vesicle selectively accumulate in the germinal vesicles of other injected oocytes. We conclude from these results that nuclear proteins will enter injected nuclei in oocytes and therefore gain access to chromosomes whose genetic activity they may regulate.

Applications and prospects

The experimental system described can be used to test the gene-regulating activity of purified macromolecules which do not readily enter cells. High resolution two-dimensional gels and subsequent peptide analyses of separated proteins provide a sensitive test for changes in the activity of many different genes in the injected nuclei. Macromolecules whose effect is to be investigated can be injected into oocytes with the test nuclei. Oocytes may then be cultured for several days, so that the injected proteins may enter nuclei and associate with genes. Changes in gene activity can then be measured by subsequent labelling and analysis of newly synthesised RNAs or proteins.

It is very desirable to study changes in the activity of genes in injected nuclei, rather than changes in the activity of oocyte genes; this is because changes in the transcriptional activity of oocytes genes would be hard to detect over the very high background of proteins being synthesised from preformed, stable mRNA of which there is large stock in oocytes. The major advantages of this living cell system over cell-free systems with isolated nuclei are the very long persistence of transcription (for several weeks) and the more natural circumstances in which presumed regulatory molecules can function. The special advantage of frog oocytes over other living cell assay systems, such as injectable fibroblasts[33] or fused vesicles[34], is that sufficient label is incorporated by a few oocytes (importantly, a few injections) for a detailed biochemical analysis of newly synthesised RNAs or proteins. For each protein analysis described in this article, we have used material from five or less oocytes.

In addition to using *Xenopus* oocytes as a living test tube in which to test molecules regulating transcription, two directions in which this type of work may be fruitfully pursued are the following. One is to try to replace whole nuclei by chromatin or purified DNA, the efficient transcription of which might substantially increase the information obtainable from chromatin reconstitution experiments. The introduction of purified macromolecules into living frog oocytes and eggs has given encouraging results in preliminary investigations[35,36]. The other is to develop a system in which inactive genes can be made active in experimentally analysable conditions. This might be achieved by injecting into oocytes somatic nuclei from a tissue in which oocyte-active genes are repressed. The activation of such genes could be seen if nuclei from one strain or species were transplanted into oocytes of another in which the two-dimensional distribution of proteins is clearly different.

We thank Drs R. A. Laskey and W. C. Russell for help with experiments involving adenovirus. We also thank Drs R. A. Laskey and J. S. Knowland for advice, and Miss J. Price for assistance. E. De R. is supported by a CNICYT (Argentina)–Royal Society exchange programme and by a fellowship of the Jane Coffin Childs Memorial Fund for Medical Research.

Received December 22, 1975; accepted February 6, 1976.

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Developmental shifts in the synthesis of heterogeneous nuclear RNA classes in the sea urchin embryo

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Three molecular classes with different properties can be distinguished in the heterogeneous nuclear RNA (HnRNA) of sea urchin embryos. The relative proportions of these classes, which we have previously designated α, β and γ HnRNA, undergo marked changes during development from morula to early gastrula. Such changes suggest the existence of more than a single HnRNA function, as well as specific roles for different HnRNA classes in embryonic development.

THE functions of heterogeneous nuclear RNA (HnRNA) may well be elucidated by investigating the involvement of HnRNA in embryonic development. Such an approach is made promising by our demonstration that HnRNA in the sea urchin embryo is not a uniform class of molecules, but is comprised of distinct molecular types[1]. In the study reported here we asked whether development is characterised by shifts in the synthesis of these distinct molecular types of HnRNA.

Early sea urchin development involves (1) the rearrangement of the cells of the morula to form the hollow sphere of the early blastula; (2) rotation of the ciliated blastula inside its ferti-

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lisation membrane; (3) lysis of the membrane to yield a hatched blastula; (4) migration of mesenchyme cells into the blastocoel, and (5) invagination to produce a gastrula. The early stages (1–3) do not seem to depend on the transcription of the embryonic genome, in so far as development to the blastula stages can proceed normally when RNA synthesis is blocked[2]. In spite of the appearance of newly synthesised mRNA in polyribosomes[3,4], the proteins synthesised during this period are minimally different from those made in the absence of RNA synthesis, that is, exclusively from maternal RNA templates[5]. This apparent absence of an immediate influence of morula and blastula transcription does not exclude an effect on later morphogenesis. Indeed, there are intriguing critical periods during early development, in which a transient block on transcription[6,7] exerts a morphogenetic influence only later, just before or after gastrulation. Thus early stage RNA transcripts may be more important for later development than for early stage protein synthesis. Our fractionation of HnRNA into molecular types may prove useful for analysing the nature and significance of these early RNA transcripts.

Table 1 Comparison of the percentage of molecules binding to poly(U) in HnRNA prepared from mixed and unmixed lysates of embryos at various stages

	Poly(U) filter binding					
	Experiment 1		Experiment 2		Experiment 3	
Developmental stage	Early blastula ^{3}H	Mesenchyme blastula ^{14}C	Morula ^{3}H	Mesenchyme blastula ^{14}C	Morula ^{3}H	Mesenchyme blastula ^{14}C
Method of preparation						
Mixed lysate	25	37	40	36	22	28
Individually prepared	20	39	40	36	27	39

Early blastula and morula embryos of *Lytechinus pictus* were labelled for 60 min with ^{3}H-uridine, and mesenchyme blastula embryos were labelled for 60 min with ^{14}C-uridine. HnRNA was prepared from a portion of each batch of embryos. Immediately after lysis of embryos, lysates of early blastula and mesenchyme blastula embryos, and morula and mesenchyme blastula embryos were mixed in 5:1 portions in each case. Nuclei and HnRNA were then prepared from each of these mixed lysates. Preparation of intact nuclei from embryos at different stages of development requires different lysis conditions because of large changes in nuclear volume during early embryogenesis[18]. Embryos were washed with 1 M dextrose, suspended in 30–60 volumes of 2 mM $MgCl_2$, and then passed through a hypodermic needle[1], except that an 18-gauge needle was used for morula (4 h), 20 for early blastula (7 h), 21 for mid-blastula (10 h), 22 for rotating blastula (13 h), and 23 for hatched blastula (16 h) and mesenchyme blastula (21 h). Nuclei were purified as before[1]. Electron microscopy revealed that some ribosomes remained attached to these nuclei. A small amount of rRNA of apparent cytoplasmic origin could also be detected when HnRNA preparations were sedimented. This rRNA can be eliminated by pelleting nuclei in a solution containing 0.005 M EDTA in addition to the 1% Triton X-100 solution previously described[1]. Preparation of HnRNA and the method of binding to poly(U) filters have also been described[1]. RNA was assayed as uridine incorporation in alkaline-labile, acid-precipitable material.

The molecular classes that we have characterised and quantified in the sea urchin embryo[1] have been defined as follows: (1) αHnRNA contains internal oligo$(A)_{25}$ and oligo$(A)_{12}$ stretches, (2) βHnRNA contains a 3′ terminal poly$(A)_{175}$ segment and internal oligo$(A)_{12}$ stretches and (3) γHnRNA lacks both poly$(A)_{175}$ and oligo$(A)_{25}$. These molecular classes have characteristic mean S values of 37, 30 and 36S, respectively, and are apparently not artefactually derived from larger molecules. The study reported here shows that the βHnRNA, which probably bears a precursor relationship to polyadenylated mRNA, is not synthesised in constant proportion as development proceeds. This observation contrasts with the constant amount of polyadenylated HnRNA in resting and growing fibroblast cells[8]. Indeed, the relative rates of synthesis of all three molecular types undergo pronounced changes as a function of embryonic development. Such compositional changes in HnRNA reinforce our concept that HnRNA molecules, in addition to differing in specific nucleotide sequences, fall into classes which are very likely responsible for separate physiological functions.

Integrity of HnRNA from early stages

Unambiguous measurements of changes in the percentages of α, β and γHnRNA during early development depend on an assurance of the integrity of HnRNA extracted at each embryonic stage. Mixing experiments were performed to test for the absence of miscible (soluble) degradative agents. The integrity of HnRNA can be monitored by binding mixed preparations to poly(U), in so far as the amount bound would be reduced by the cleavage of portions of the molecule from oligo(A)- or poly(A)-containing HnRNA segments. Early blastulae and mesenchyme blastulae with different isotope labels were lysed, portions of the lysates were mixed, and the poly(U) binding of HnRNA mixtures was compared with that of the HnRNAs prepared from the original lysates (experiment 1, Table 1). The percentages of poly(U) filter-bound HnRNA from individually prepared HnRNAs clearly differed for the two stages, 20–25% for the early blastula and 37–39% for the mesenchyme blastula. We conclude that the lower value for the earlier stage is not attributable to the presence of miscible degradative agents, because the value of the mesenchyme blastula HnRNA was not significantly reduced as a result of having been prepared from a mixed lysate.

This mixing protocol can be used to choose between divergent values, such as the 40 and 22% (experiments 2 and 3, Table 1) for two preparations of morula HnRNA. The lower value can probably be attributed to the action of degradative agents, since mixing the low binding morula HnRNA with mesenchyme blastula HnRNA reduces the binding value of the latter from 39 to 28% (experiment 3). In contrast, the morula HnRNA of high value (experiment 2) does not affect the mesenchyme blastula HnRNA value. The poly(U) binding of morula HnRNA is then approximately equal to that of mesenchyme blastula HnRNA. In our conditions of preparation, enzymes able to degrade HnRNA in exogenous nuclei may in certain cases be active at the morula stage but not at the early blastula stage or mesenchyme blastula stage. The integrities of HnRNAs were also compared by sedimenting total HnRNA in denaturing solvent[9]. Slightly higher S values were obtained for HnRNA of 13-h rotating blastulae (34–36S) compared with 20-h mesenchyme blastulae (28–30S). The lower mean S value for total HnRNA in the later stage embryo may be attributed to an increase in the proportion of βHnRNA, which possesses the smallest mean S value of the three HnRNA classes[1].

Developmental changes in HnRNA composition

The HnRNA which binds to poly(U) filters consists of at least two general classes, αHnRNA containing oligo(A) and βHnRNA containing poly(A)[1]. These components can be resolved by elution with a gradient of logarithmically increasing concentration of the denaturing solvent formamide and decreasing salt concentration, the αHnRNA being eluted at 15% and the βHnRNA at 40% formamide. These observations were made with HnRNA from 13-h prehatched, rotating blastulae[1], and the elution profile for this stage has been re-

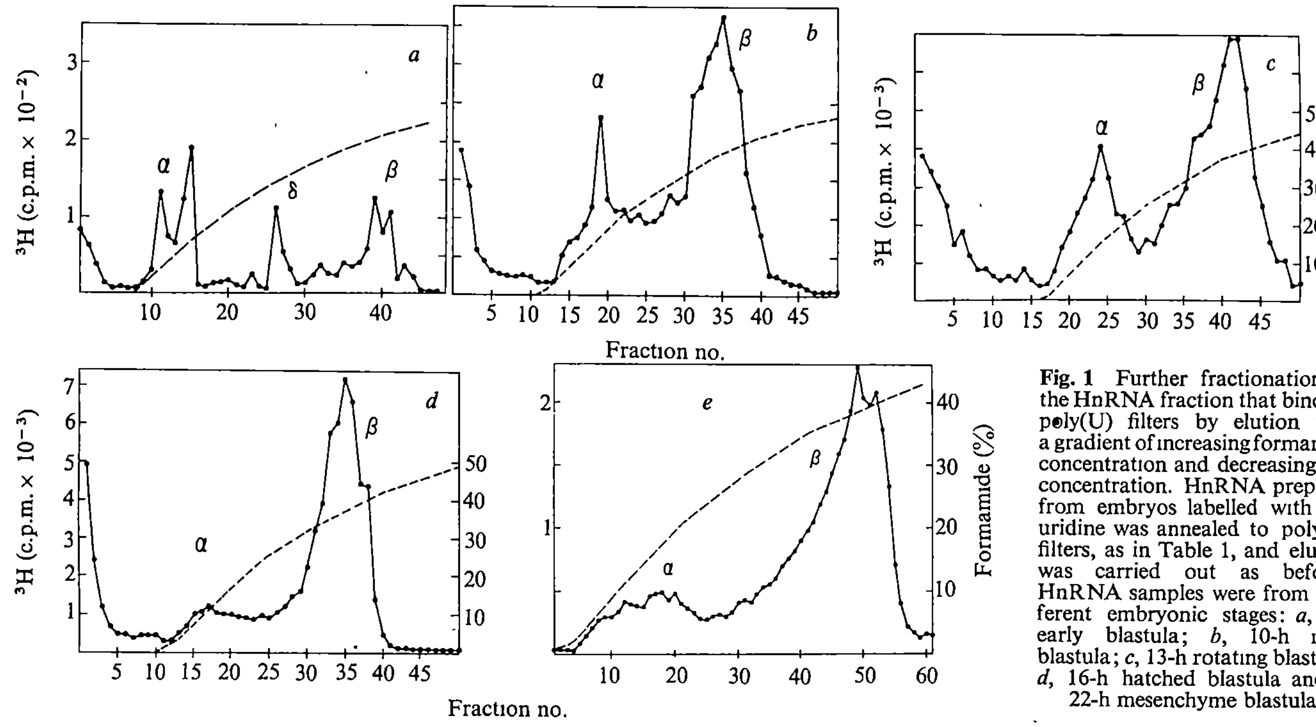


Fig. 1 Further fractionation of the HnRNA fraction that binds to poly(U) filters by elution with a gradient of increasing formamide concentration and decreasing salt concentration. HnRNA prepared from embryos labelled with ^{3}H-uridine was annealed to poly(U) filters, as in Table 1, and elution was carried out as before[1]. HnRNA samples were from different embryonic stages: *a*, 7-h early blastula; *b*, 10-h mid-blastula; *c*, 13-h rotating blastula; *d*, 16-h hatched blastula and *e*, 22-h mesenchyme blastula.

produced in Fig. 1*c*. Here and in earlier stages (Fig. 1*a* and *b*) the αHnRNA represents a substantial proportion, at least 30%, of the labelled HnRNA bound to poly(U). In later blastula stages (Fig. 1*d* and *e*) the proportion of αHnRNA is considerably diminished, with the βHnRNA very much predominant. The ratio of βHnRNA to αHnRNA increases some 2–4-fold during development from early to late blastula stages. Details of the elution profiles reveal that the αHnRNA tends to be resolved into two peaks. Another peak, eluted at 30% formamide, is especially evident in the 7-h early blastula (δ, Fig. 1*a*). The component can be made to bind partially (~50%) to oligo(dT) cellulose[9]. This behaviour resembles neither that of αHnRNA, which does not bind to oligo(dT), nor that of βHnRNA, which binds completely to oligo(dT)[1]. Further characterisation of the δ component is in progress (unpublished results of L.M.D.)

We have shown previously that the α, β and γ classes of HnRNA could be separated and quantitated by virtue of their binding properties to oligo(dT)–cellulose and poly(U) filters[1]. βHnRNA binds oligo(dT)–cellulose through its poly(A) segment. αHnRNA binds through oligo(A) segments to poly(U), together with βHnRNA; however, it does not bind to oligo(dT)–cellulose. Thus the percentage of αHnRNA is obtained by subtracting the percentage bound to oligo(dT)–cellulose (βHnRNA) from the percentage bound to poly(U) filters (α + β HnRNA). γHnRNA will not bind to poly(U) filters.

Analyses of HnRNA preparations from early blastula (7 h), rotating blastula (13 h) and mesenchyme blastula (21 h) embryos exemplify the measurements of differential binding (Table 2). The HnRNA from each stage was separated into fractions bound and not bound to oligo(dT)–cellulose, and these fractions were further examined for bindability to oligo(dT)–cellulose or poly(U) filters (Table 2). For each of the embryonic stages the bound fraction (11%, 16% and 33% for the respective stages above) was almost completely retained on

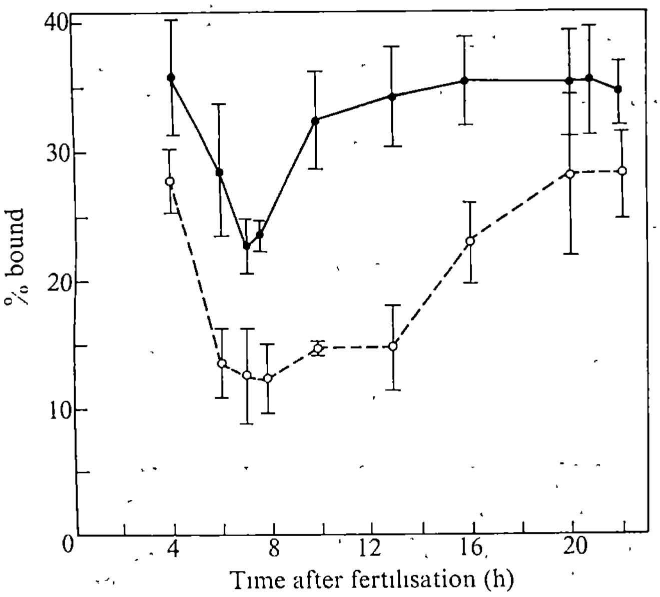
Table 2 Binding measurements of RNA and oligo(dT)–cellulose-fractionated RNA to oligo(dT)–cellulose and poly(U) filters

RNA tested	% Total HnRNA bound to oligo(dT)–cellulose	% Total HnRNA bound to poly(U) filter	% Not bound
HnRNA from 7-h early blastula	11 (βHnRNA)	24	76 (γHnRNA)
Rerun HnRNA bound to oligo(dT)	10	10	
Rerun HnRNA not bound to oligo(dT)	<0.1	14 (αHnRNA)	
Sum of rerun HnRNA		24	
HnRNA from 13-h blastula	16 (βHnRNA)	36	64 (γHnRNA)
Rerun HnRNA bound to oligo(dT)	16	15	
Rerun HnRNA not bound to oligo(dT)	<0.1	15 (αHnRNA)	
Sum of rerun HnRNA		30	
HnRNA from 21-h mesenchyme blastula	33 (βHnRNA)	37	63 (γHnRNA)
Rerun HnRNA bound to oligo(dT)	32	31	
Rerun HnRNA not bound to oligo(dT)	<0.1	5 (αHnRNA)	
Sum of rerun HnRNA		36	

HnRNA was from embryos labelled for 60 min with ^{3}H-uridine. Assays of binding to poly(U) filters and oligo(dT)–cellulose have been described previously[1]. RNA was assayed as uridine incorporation in alkaline-labile, acid-precipitable material in all fractions. See text for definitions of α, β and γHnRNA. The 14% in α and the 11% in βHnRNA in the 7-h early blastula must be corrected for the approximately 3% in the δ component, estimated from Fig. 1*a*. Since approximately half of the δ component binds to oligo(dT) together with βHnRNA and the other half is not bound, in company with αHnRNA (unpublished results of L.M.D.), the corrected values are approximately 12% in αHnRNA and approximately 9% in βHnRNA.

a subsequent passage through oligo(dT)–cellulose or poly(U) filters, and the unbound fraction remained unbound after a second passage through oligo(dT)–cellulose. Repassage of the unbound fraction through poly(U) filters, however, resulted in the binding of part of this fraction to an extent representing 14%, 15% and 5% of the respective total HnRNAs. At each stage this additional HnRNA plus the HnRNA bound to oligo(dT)–cellulose, was approximately equivalent to the percentage of the total HnRNA bound originally by poly(U) filters. Therefore, HnRNA that is bindable to poly(U) filters comprises two classes at each stage, distinguishable by their binding properties to oligo(dT)–cellulose. Quantitative differences are observed in the content of α, β and γHnRNA classes in the 7-h morula, 13-h rotating blastula and 21-h mesenchyme blastula as follows: αHnRNA comprises 12%, 15% and 5% at these successive stages, βHnRNA is 9%, 16% and 33% and γHnRNA represents 76%, 64% and 63%, respectively.

Fig. 2 Labelled HnRNA from various stages of development bindable to oligo(dT)–cellulose columns and poly(U) filters. Embryos at various stages of development were labelled with 10–100 μCi ml^{-1} of ^{3}H-uridine for 60 min. HnRNA prepared from these embryos was bound to oligo(dT)–cellulose (○) or poly(U) filters (●). Each symbol represents the mean and standard deviation derived from determinations on 5 to 18 batches of embryos for each stage. The points represent the average stage at which the 60 min incubations were terminated.



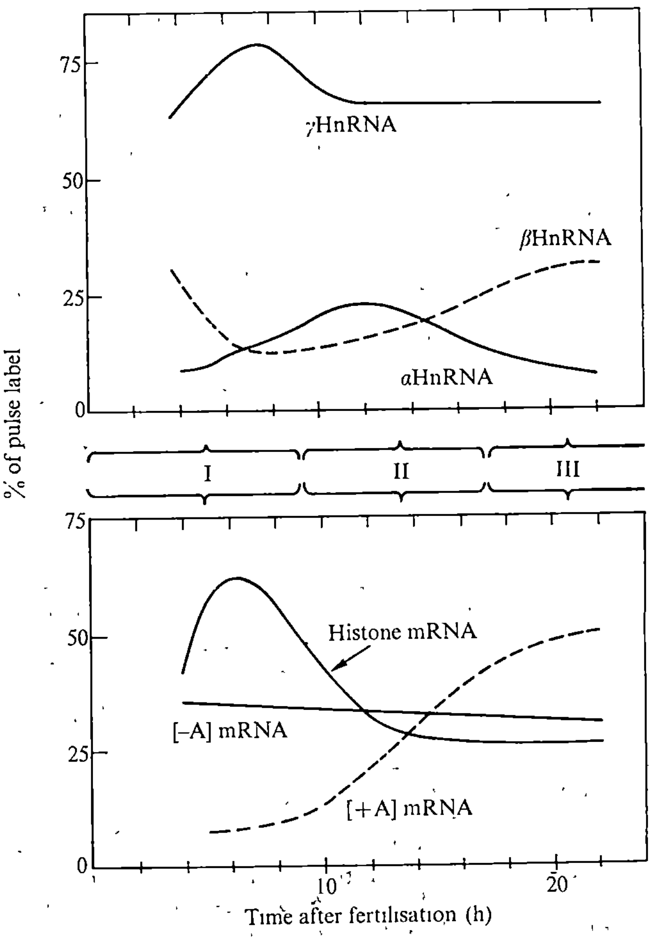


Fig. 3 Changes in the percentage incorporation in HnRNA and mRNA classes during development. The curves in Fig 2 are used to generate continuous developmental curves of α, β, and γHnRNA. αHnRNA is measured as the difference between poly(U) filter binding and oligo(dT)–cellulose binding. βHnRNA is that which binds to oligo(dT)–cellulose, and γHnRNA is that not bound to poly(U). Included also are the curves for relative incorporation of uridine in mRNA (non-4S and non-ribosomal RNA in polyribosomes) for 9S putative histone mRNA, poly(A)-containing mRNA ([+A] mRNA) and mRNA lacking poly(A) ([–A] mRNA), according to Nemer[11]. Between the developmental curves, three characteristic developmental periods are approximated: I, an early period of rapid cell division of the morula and early blastula; II, a middle period of relative quiescence that includes rotation of the embryo and hatching from its membrane. During this period the embryo is uniquely sensitive to actinomycin treatment, in so far as gastrulation is later inhibited[7]. III, A period of development of the mesenchyme blastula leading into gastrulation.

The quantitative changes in HnRNA composition, particularly in the relative incorporations in α and βHnRNA, can be evaluated by two methods, either graphically estimating components eluted by formamide from poly(U), as in Fig. 1, or the differential binding to oligo(dT)–cellulose and poly(U) filters, as exemplified by Table 2. Measurements can be made easily on a multitude of samples by the method of differential binding. The percentages of labelled HnRNA bound to oligo(dT)–cellulose and poly(U) filters are recorded in Fig. 2 for numerous batches of embryos from the 4-h morula to the 22-h mesenchyme blastula stage. The amounts of HnRNA bound to these two polymers differ widely from each other and fluctuate differently during development. The fraction bound to oligo(dT)–cellulose is a direct measure of the βHnRNA, whereas the difference function between these two curves represents αHnRNA. The γHnRNA fraction is not bound to poly(U) filters, and thus its relative incorporation during development is represented by the inverse of the curve for binding to poly(U) filters. Continuous curves for each of these HnRNA components, drawn from these data are given in Fig. 3. They seem to demonstrate three periods during early embryogenesis, each characterised by a distinctively different pattern of HnRNA synthesis. During the first period, from cleavage to early blastula (to approximately 9 h), relative incorporation in γHnRNA, the most prevalent component, attains a maximum value. HnRNA composition during the early part of this period is uncertain, due to the variable degradation of 4-h morula HnRNA noted in Table 1. Futhermore, the relative contributions of α and βHnRNAs are slightly lower than represented by the method of differential binding (Fig. 2), due to the presence of the δ component (Fig. 1*a* and *b*). The relative amounts of α and βHnRNA were therefore quantified from elution profiles, such as those in Fig. 1. They have similar amounts of incorporation at the 7-h early blastula stage and their sum displays a minimum value inversely related to the γHnRNA maximum. At stages beyond 9–10 h, the definition and quantitation of α and βHnRNA by the method of differential binding is fairly unambiguous. In the second period, from the mid-blastula (10 h) to hatched blastula (16 h), αHnRNA reaches a maximal value and declines; γHnRNA has a constant value throughout this and the next period;

βHnRNA increases gradually. In the third period, involving development through mesenchyme blastula (22 h), βHnRNA increases sharply and αHnRNA continues to decline.

HnRNA changes and cellular physiology

These results show that different molecular types of HnRNA can be distinguished. Furthermore, relative incorporation in the distinct HnRNA classes, which we have designated α, β and γ, varies as a function of embryonic development. Control experiments indicate that this variation does not reflect preparational artefacts of degradation or contamination. Considering that the 60-min labelling time is at least four times longer than the turnover time for total nuclear RNA[10], these changes in relative incorporation can be regarded as changes in steady-state populations rather than metabolic turnover differences among the classes at different stages. In addition, they may reflect precursor–product relationships among different nuclear and cytoplasmic RNA classes. The nature and timing of developmental shifts in the synthesis of each of these classes may furnish clues about their separate functions.

(1) The relative syntheses of βHnRNA and poly(A)-containing mRNA[11] increase concomitantly during development from early blastula to mesenchyme blastula (Fig. 3). These changes might be expected to support changes in protein synthesis that accompany extensive and obvious morphogenetic differentiation, especially the appearance of the three germ layers. The changes in HnRNA may be related to changes in mRNA synthesis, involving selective transcription, nuclear poly(A) polymerase activity[12] and the processing of mRNA precursor molecules.

(2) The relative rate of synthesis of γHnRNA attains a peak value at the early blastula stage, a time of maximal rate (per embryo) of DNA and histone synthesis[13], and of the relative synthesis of putative histone mRNA (Fig. 3). This lends credence to the proposition that histone mRNA, which lacks poly(A), is derived from precursors in the poly(A)-deficient γHnRNA class. Only a small portion of the γHnRNA, however, can be involved, since γHnRNA continues to represent the most prevalent HnRNA component even in later stages, when histone and histone mRNA syntheses are much less prominent. The bulk of γHnRNA seems to be involved in processes other than histone mRNA synthesis.

(3) The relative incorporation in αHnRNA peaks at the mid-blastula stage, the period between the beginning of rotation within the fertilisation membrane and hatching. This seems to be an interval of relative quiescence, following the period of intense cell division (and γHnRNA synthesis) and preceding the events leading to gastrulation (including increased βHnRNA synthesis). But it is during this period only that inhibition of transcription results in a block of gastrulation, which occurs much later[6,7]. Thus, this is a critical period of RNA synthesis for the occurrence of a complex future developmental event. There is no evidence for or against the involvement of any individual RNA class. If, however, we consider the developmental changes in the three classes of HnRNA and the three (not necessarily homologous) classes of mRNA in Fig. 3, we must conclude that only αHnRNA attains a characteristic prominence during this critical period. The circumstantial evidence in this chronology suggests the novel hypothesis concerning the role of transcription in the programming of development: that a special class of HnRNA molecules (αHnRNA) functions to predetermine the outcome of later developmental events (gastrulation).

The involvement of αHnRNA could entail the recognition of distinct chemical properties[1], consistent with the operation of developmental signals. It remains to be determined whether or not translatable nucleotide sequences are present in αHnRNA. If this were so, then the critical function of αHnRNA might be to supply templates for immediate or future translation. Alternatively, the regulatory function of such an HnRNA class might not depend on its giving rise to mRNA, but on its intrinsic properties. Theoretically, HnRNA molecules or their derivatives might activate and coordinate gene activity[14], influence mRNA translation[15], or interact with chromatin and influence chromosomal structure[16,17].

We thank Melissa Graham for technical assistance. This research was supported by grants and a fellowship (to L.M.D.) from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania.

Received October 23, 1975, accepted January 21, 1976

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letters to nature

Test of pulsar acceleration mechanisms

An elegant mechanism to explain the high velocities of pulsars has been proposed by Harrison and Tademaru[1,2]. Based on the observations that the interpulses seen in some pulsars are located asymmetrically between the main pulses, they assume that pulsar magnetic fields arise in general from off-centred dipoles. The asymmetric radiation reaction produced by such an inclined off-set rotating dipole accelerates the pulsars in a direction parallel to their spin axes and gives the observed velocities. Any verification of the above hypothesis hinges on the possibility of determining, and comparing, the angle projected by the spin axis on the sky S with the observed direction of motion of the pulsar V. According to Harrison and Tademaru's model[1,2], these directions would coincide ($V = S$). We wish to point out here that a measure of the spin axis projected direction is given by the intrinsic angle of polarisation of the radio emission at the centre of the integrated pulse profile, and that these polarisation data do not support the hypothesis in most cases.

It may be remarked here that in those pulsars from which asymmetrically located inter-pulses are seen, this mechanism will be weak, if operative at all, since the magnetic dipole is almost at right angles to the rotational axis. Furthermore, asymmetrically located inter-pulses cannot result from merely

Table 1 Data for $\varphi_I - V$ for pulsars (See text for details)

Pulsar	SNR	V		ref.	φ_I	ref.	$\varphi_I - V$
0329+54	CTB13 ??	293±15	Sc	7	80±10	10	33±18
		126±8	PM	7			46±13*
0525+21	S147?	203±7	D		40±10	10	17±12
0531+21	Crab	294±8	PM	5	145±20	10	31±22
0611+22	IC443	274±10	D		150±70	16	56±71
0823+26		158±3	PM	7	73±6	17,16	85±7
0833−45	Vela X	234±15	D	†	47±10	18	7±18
0834+06		−19±10	PM	7	105±80	10	86±81
1133+16		0±15	PM	6	93±10	14,10	87±18
		−10±4	PM	7			77±11
1237+25		75±4	PM	7	0±15	10	75±15
1749−28	W28?	200±5	D		9±8	14,16	11±10
1929+10	CTB72?	22±8	D		64±6	14,10	42±10
2016+28		9±13	PM	7	100±50	10	89±51
2021+51	HB21?	287±5	D		50±7	15,16	57±9

*Adopted value.
†Using maps at 408 MHz and 635 MHz.

offsetting a dipole field; the direction of emergence of the field lines from the surface must be modified such that these directions for the two poles are not at 180°.

Competing acceleration mechanisms predict different relations between V and S. For example there is evidence that in visual binaries the spin axes of the individual stars are preferentially aligned perpendicular to the orbital plane[3]. The sling shot acceleration mechanism for runaway stars proposed by Blaauw would then produce motion perpendicular to the spin axes of the stars ($V = S + 90°$), if a similar alignment takes place in close binary systems producing pulsars. On the asymmetric supernova explosion hypothesis discussed by Shklovsky and Michel[4], it is not clear if a preferred direction of ejection relative to the spin axes would be expected.

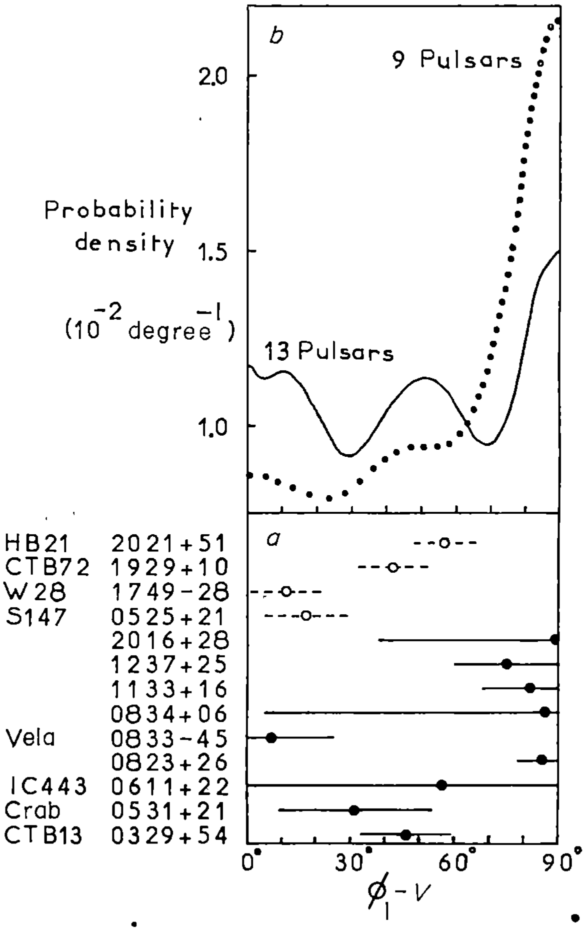
The angle V can be found from proper motion measurements[5–7], from the angular displacement of pulsars from their associated supernova remnants, or from interstellar scintillation observations[7]. We have collected the available measures of V and φ_I, the intrinsic angle, and on this basis we will assess here the relative importance of the various proposed acceleration mechanisms.

We make two general assumptions: firstly, as in most theories of pulsar radio emission, we attribute the systematic sweep of the plane of the average polarisation to the changing aspect of the projected magnetic field in the radiating region as the pulsar rotates, and assume that there is a constant relation between the projected field direction and the plane of polarisation. Secondly, we assume that at pulse maximum, there will be a constant difference between the projected magnetic field direction (and hence the plane of polarisation) and the projected direction of the spin axis, independent of other parameters such as velocity of rotation or inclination of the spin axis to the plane of the sky. In such cases $\varphi_I = S + k$ can be used as a measure of the projected spin axis.

There is evidence in the case of the Crab Nebula that the constant k is zero. If the magnetic field in the nebula as a whole is wrapped toroidally about the pulsar spin axis[8], then measurements[9] of the optical polarisation of the nebula imply a spin axis at position angle 140°. This is in close agreement with the intrinsic position angle[10] of the pulsar radio emission (145 ± 20°). It may be noted that the value $k = 0$ is just that predicted by those polar cap models[11] in which the radio emission comes from (coherent) curvature radiation. Thus, in summary, for many pulsar models $\varphi_I = S + k$, and a preferred value of $\varphi_I - V$ would imply a directed ejection of pulsars from supernovae. If, as seems likely, $k = 0$, then a preferred value of $\varphi_I - V = 0°$ is expected from Harrison and Tademaru's hypothesis, and $\varphi_I - V = 90°$ for the sling shot or runaway star mechanism.

Table 1 gives the available values of $\varphi_I - V$, expressed |180°|. The supernova that has been associated with each pulsar is indicated in column 2, with ? to indicate doubtful cases. The type of observation leading to the adopted value of V (column 3) is shown in column 4. Sc denotes an interstellar scintillation measurement, PM a measured proper motion

Fig. 1 *a*, Plot of the difference ($\varphi_I - V$) between the intrinsic position angle of polarisation φ_I at the centre of the pulse profile and the direction of proper motion V. Values dependent on doubtful associations are dotted *b*, The differences $\varphi_I - V$ of Fig. 1*a* plotted as a probability distribution, taking into account the different weights of the various measurements as given in column 8 of Table 1 ——, All data; . . . . , with uncertain supernova remnant associations excluded.



(either by pulse-timing or interferometry), and D indicates a measured displacement of the pulsar from the centre of the associated supernova remnant. Pulsar positions were taken from the compilation of Manchester and Taylor[12], and the supernova remnant positions were obtained from published radio maps. The source of the values of φ_{I} (column 6) is indicated in column 7. In some cases, lower errors in extrapolating to zero wavelength have been obtained by taking high frequency observations[13–15] and correcting for Faraday rotation by using published Rotation Measures derived independently from low-frequency observations[10,16]. For these pulsars two references are given in column 7. For PSR2021+51 a position angle of polarisation of 38 ± 5° at 1,665 MHz has been taken from ref. 15 and corrected using a Rotation Measure[16] of $-6.5\ \mathrm{m}^{-2}$.

The values of $\varphi_{\mathrm{I}} - V$ (in the range 0°–90°) from Table 1 are displayed in Fig. 1*a* and are shown as a probability distribution in Fig. 1*b* in an attempt to allow for the different weights of the various measurements. There is a preponderance of values of $\varphi_{\mathrm{I}} - V$ close to 90° in the full curve containing all the data. The peak in probability density at $\varphi_{\mathrm{I}} - V = 0$ in Fig. 1*b* comes largely from data for pulsar–supernova associations which are not very well established, S147, W28, HB21 and OTB72. If these data are not included (dotted line), then the preferred direction $\varphi_{\mathrm{I}} - V = 90°$ becomes much more pronounced. If we now apply the condition that the polarisation and the spin axis are related as predicted by the curvature radiation polar cap models, and supported by observations of the Crab Nebula and the pulsar PSR0531+21, this correlation indicates that the acceleration mechanism proposed by Harrison and Tademaru is clearly not dominant.

The distribution in Fig. 1*b* seems to favour a 'sling shot' or similar mechanism or a 'spin-aligned' ejection of pulsars from supernovae. The scatter of values about 90° can be attributed to observational errors, the residual influence of the Harrison and Tademaru mechanism, or more likely to random effects of which at least two can be envisaged. Firstly, any significant proper motion of the parent star before the supernova explosion will make a contribution to the motion of the pulsar. Secondly, an intrinsic scatter of the spin angular momentum of the parent star about the normal to its orbital plane can be expected on the sling-shot hypothesis.

Although the dotted line distribution of Fig. 1*b* is clearly peaked at 90°, we note that for the two pulsars whose association with supernovae is least questioned, (the Crab and Vela), $\varphi_{\mathrm{I}} - V$ is very close to zero and in fact responsible for the tail of the distribution. This raises the possibility that there might be a difference between pulsars associated with supernovae and those not. Support for the possibility that not all pulsars are created in supernova explosions is provided by the case of the binary pulsar PSR1913+16 which has no observable associated remnant, although it is the second fastest pulsar known[17]. Perhaps the asymmetric radiation reaction mechanism operates only on pulsars created in supernovae, because the rotational speeds attained initially might be very much higher than those for pulsars created through accretion in binaries, for example.

We thank Dr A. G. Lyne for supplying the data on proper motions[7] before publication.

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Received November 12, 1975, accepted January 28, 1976.

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Angular momentum diffusion and the initiation of cyclones

IT is possible to explain[1,2] the intensification of a cyclone vortex once the stream lines in a weak depression close around a core of higher vorticity, but it is less clear how the process starts. Scorer[3] proposed that convection might diffuse angular momentum within a field of initially uniform rotation to localised regions, thereby concentrating cyclonic vorticity. Experiments failed to demonstrate the effect[4–7], and although strong vortices could be produced through the breaking of inertial waves[8], the effects were open to misinterpretation. We present here new experimental results showing that first, angular momentum or relative vorticity can be diffused at a rate comparable with the diffusion of relative velocity (turbulent 'viscosity'). This distinctive property seems to be intrinsic to weak turbulence in rotation, and can be parameterised very simply. Second, such diffusion occurs only when the turbulence is sufficiently weak to be constrained by rotation to an essentially two-dimensional structure. This might explain the failure of earlier experiments. Our results could have many applications to geophysics, including tropical cyclogenesis.

The experiments were conducted in a transparent, plane-ended cylinder of uniform height h (22.8 cm) and radius a (14.8 cm) mounted axially on a turntable (with rotation rates Ω between 3 and 11 rad s^{-1}), completely filled with a homogeneous fluid (water, or a glycerine–water solution). The bottom was regularly perforated by 199 holes of 2.21 mm bore, at 2 cm spacing. Each hole connected through tubing to one of two manifolds, 49 holes at 4-cm regular spacing going to one and the remainder to the other (Fig. 1*a*). Fluid (with a few polystyrene beads suspended in it for visibility) was pumped between the manifolds to induce a radially uniform, regular, small scale mixing pattern without mean advection in the radial plane. The convective geometry was varied by closing some jets or by pump reversal. Figure 1*b* shows array geometries. In some experiments using array III, a false top was put in the container to vary h.

Horizontal velocities in the central third of the cylinder were measured photographically. Without rotation, the jets caused the paths shown in Fig. 2*a*. Figure 2*b* shows the effect of rotation with the same jet conditions. Particle concentrations reveal intense vortices, always cyclonic, drifting slowly within the rotating frame, apparently through mutual interaction or by weak relative rotation of the fluid body (usually $<0.01\ \Omega$), independently of their position relative to the jets. The structure was usually well established within 30 rotation periods after pumping started. Once formed, vortices usually kept their identity. They merged occasionally, but others would form to keep the population constant. The vortices aligned themselves parallel to the rotation axis and extended from top to bottom.

Since particle paths were roughly circular and coaxial near the vortices, a mean relative circumferential velocity v could be measured as a function of local radius r from the origin of the vortex centre. This was expressible as an absolute azimuthal velocity $V = \Omega r + v$ in an irrotational frame.

Randomly chosen vortices were measured for a variety of conditions of Ω, h, pumped volume flux M, number of jets N, and kinematic viscosity ν. The profile at V as a function of r plotted logarithmically, had a similar shape for all results

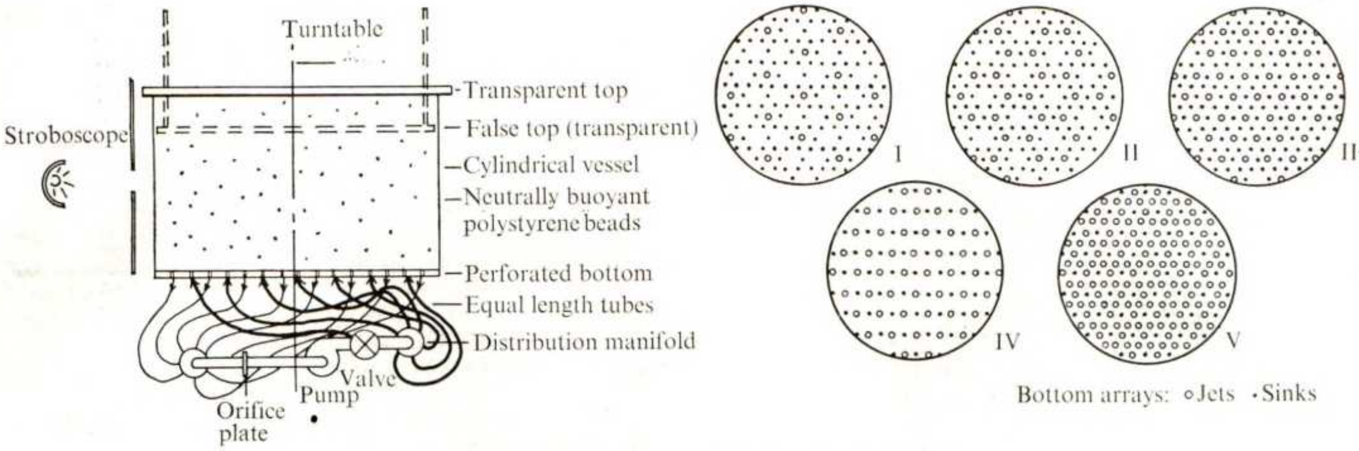

Fig. 1 Experimental arrangement and pumping arrays.

and thus could be aligned, within experimental accuracy, to a 'similar' profile (Fig. 3*a*).

$$V/Q = f(r\Omega/Q) \tag{1}$$

when scaled by a suitable parameter Q having the dimensions of velocity. Q was found to depend linearly on $w\rho/\delta$, where w is the vertical convective velocity scale ($= M/\pi a^2$), ρ is the r.m.s. jet spacing ($= aN^{-1/2}$) and δ is the scale of the viscous boundary layer on the end walls, defined here as $(\nu/\Omega)^{1/2} + w/2\Omega$. As a limit to the magnitude of Q, coherent vortices seldom formed when

$$w_j/\Omega h > 1.15 \pm 0.05 \tag{2}$$

where $w_j = w\rho^2/a_j{}^2$ defines a mean maximum vertical velocity at jet exit and a_j is the jet radius.

Every feature of these results can be explained as follows: To conserve angular momentum in a steady axisymmetric system the torque coming from the turbulent stress must balance the momentum change caused by the radial divergence, i.e., $\mathrm{d}(Tr^2)/\mathrm{d}r = \gamma Ur\,\mathrm{d}(Vr)/\mathrm{d}r$ where T is the mean tangential stress and γ is density; the radial mean velocity U is induced by end-wall boundary layers and has magnitude $\nu\delta/h$.

At its simplest, externally imposed turbulence is characterised by a single horizontal velocity scale q. T may depend both on q and the mean flow quantities, and by dimensional considerations, 'universal' similarity profiles as in equation (1) can generally occur only when Q is proportional to qh/δ. If, through rotational constraint, the turbulence approaches two-dimensionality, horizontal and vertical velocity scales become simply related and $q \propto w\rho/\delta$, consistent with equation (2), so $Q = cw\rho/\delta$ (c is some constant) precisely as observed.

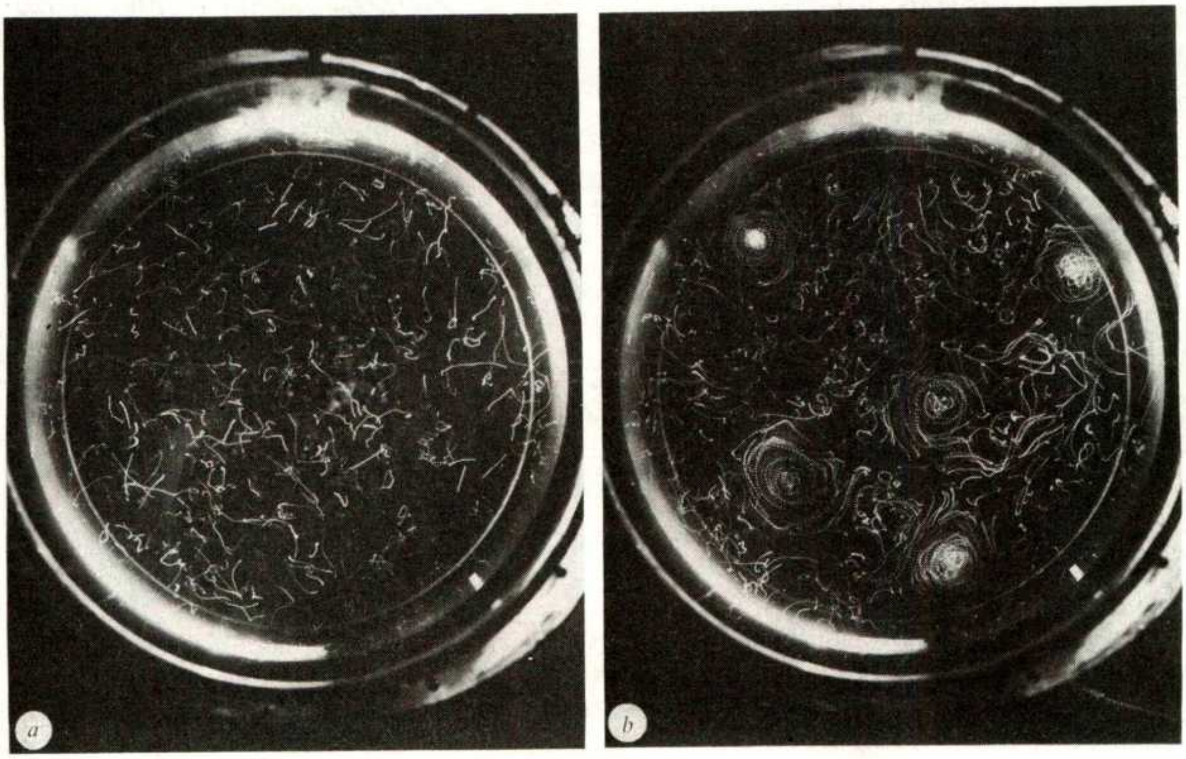

Fig. 2 Particle movements, ~1 s exposure in 50-Hz stroboscopic light by a camera mounted axially on the turntable, with array III; pumping rate for both figures is the same, corresponding to $w = 0.3$ cm s^{-1}. *a*, No rotation. Particle movements are random and short in scale. *b*, Rotation 8.24 rad s^{-1}, after pattern development. Intense cyclonic vortices, dissociated from the bottom jets, are sustained.

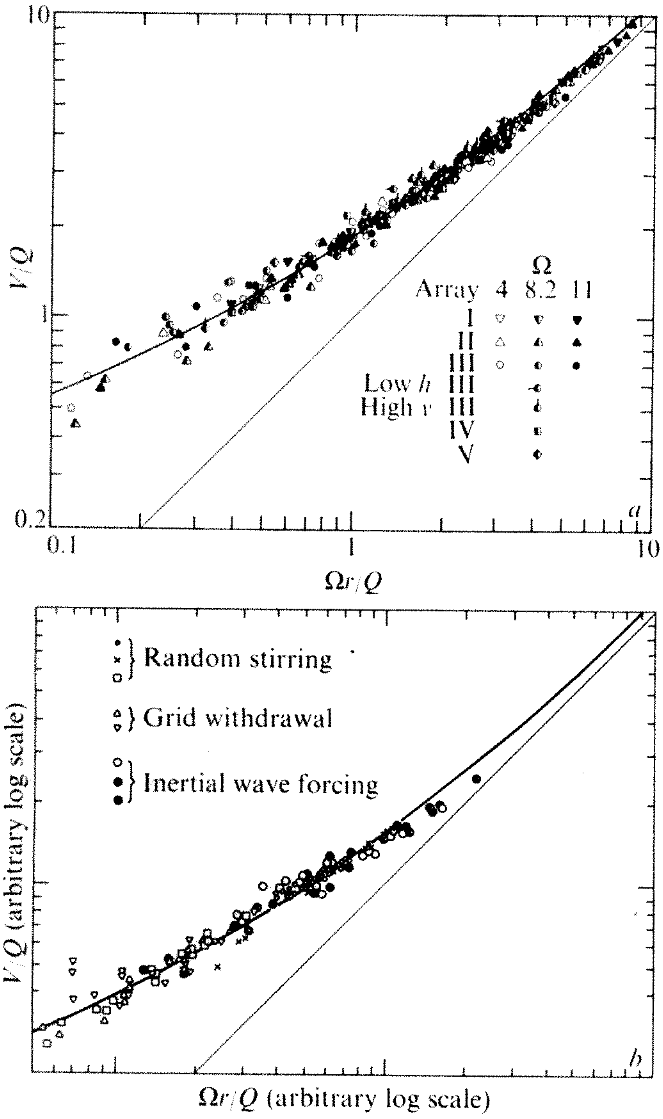

Fig. 3 Absolute azimuthal velocity V as a function of radius r from vortex core, for experimental conditions listed (logarithmic coordinates). V and r are scaled for each data set by velocity Q. Solid rotation Ω is delineated by a faint solid line. The bold line is the theoretical 'universal' absolute velocity profile arising with a constant ratio (2/3) between angular momentum diffusivity and turbulent 'viscosity'. *a*, Randomly chosen vortices with the experimental arrangement described; *b*, with alternative mixing methods: (1) after random excitation, $\Omega = 1.4$ rad s^{-1}; (2) after sudden axial withdrawal of a grid comprising 1-cm-wide battens in a 4×4 cm square plane array (performed by M. Riefman); (3) Continuous resonant forcing of inertial waves following the method of McEwan[8] (performed by W. McKimm) $\Omega = 8.2$ rad s^{-1}.

The essential influence of rotation is reflected in the law defining T, the mean tangential stress. If angular momentum were conserved during radial mixing the quantity, T would vary linearly with $r^{-1}\mathrm{d}(Vr)/\mathrm{d}r$, but in isotropic turbulence behaving like laminar viscosity, T would be proportional to $r\mathrm{d}(Vr^{-1})/\mathrm{d}r$. Between these extremes universal profiles arise only when the two terms are in constant proportion $\xi : 1$. Then equation (1), with T so specified, can be solved numerically. The curve of best fit, $\xi = 2/3$, $C = 0.82$ is shown in Fig. 3*a*. Figure 3*b* shows the fit to vortices produced after cessation of mechanical stirring, or by inertial wavebreaking as used before[8]. Agreement is uniformly good. This explanation implies that the eddies of rotationally dominated turbulence are constrained to a specific anisotropic form. This explains how the diffusion properties can be characterised by a single velocity scale. Note that positive potential vorticity[9] diffusion could not yield our profile.

The significance of the foregoing in tropical cyclogenesis is gauged by considering a disturbance in which the net atmospheric rotation is $2.5\times10^{-5}\,\mathrm{s}^{-1}$, $h = 12$ km, w is 1 cm s^{-1} and 3 cm s^{-1} between clouds at cloud base and in the deep convection zone respectively. Cloud spacing $2\rho = 20$ km and δ is ~ 200 m.

The depression would then be intensified significantly by 0.5 mbar over a 570-km radius, and the vorticity increased to $1.4\times10^{-4}\,\mathrm{s}^{-1}$ at a 10-km radius in a one-week time scale, independently of any thermodynamic advective effects. The condition in equation (2) is exceeded in active cloud columns where $w_j/\Omega h$ is ~ 10, but not in the intercloud volumes. Considering the ease with which the controlling variables can be quantified, further observation and simulation studies seem worthwhile.

Support for part of this work as Rossby Fellow at the Woods Hole Oceanographic Institution is acknowledged.

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Received November 12, 1975; accepted January 28, 1976.

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Rock volatility and aerosol composition

On the basis of recent measurements, I propose here that part of the Earth's aerosol burden comes from the volatilisation (sublimation) of the components of surface rocks, in addition to the well known man-made contribution. Calculations on a simple kinetic model indicates that vapour pressures of $\sim 10^{-11}$ atm could support the observed atmospheric concentration of copper.

Analyses of the inorganic constituents contained in aerosols and rains show a remarkable similarity in abundance patterns (Table 1). In general, the sequence of decreasing concentrations for a group of heavy metals is: Pb, Zn, Cu, Mn and Ni. The measurements cited were made in both hemispheres and on several continents, in urban and rural areas and in all seasons of the year. It is thus difficult to relate these findings to pollution sources, particularly for the heavy metals in the South Pole atmosphere[1]. The above sequence does not resemble that found in crustal rocks or soils (Table 1), where, for example, the concentration of manganese is an order of magnitude greater than that of the other four metals.

Two lines of evidence support the hypothesis that the fractionation responsible for such distributions involves a selective volatilisation. First, emission spectrographers have long been aware of the selective volatilisation of the elements and their compounds[2]. This depends on the matrix in which the elements are bound. For sulphides, oxides, sulphates, carbonates, silicates, and phosphates, the distillation rankings are similar: Pb, Zn, Cu, Mn and Ni, listed in order of decreasing volatility. This applies, however, to extrapolation from high temperatures: vapour pressures for lower temperatures have not yet been measured for this suite of metals and their compounds (there are isolated measurements of only some of the more volatile species). Also, these metals exist in nature in forms other than those used for emission spectroscopic studies. Nonetheless, the matching of the two sequences seems significant.

Kinetic theory shows that even their low vapour pressures are sufficient to account for steady state fluxes of these species

Table 1 Trace metal concentrations in aerosols, rains and crustal rocks*

	Pb	Zn	Cu	Mn	Ni
Mean concentrations in South Pole atmosphere[1] (10^{-12} g m^{-3})	630	30	36	10	
Average concentrations in aerosols collected in San Francisco Bay area[12] (10^{-9} g m^{-3})		136	50	17	
Average concentrations in US precipitation[13] (parts per 10^9)	34	107	21	12	4
Average concentrations in Tucson air[14] (10^{-6} g m^{-3})	0.47	0.15	0.41	0.013	0.021
Average concentrations in rain at Wraymires, UK[15] (10^{-6} g l^{-1})	39	85	23	8	6
Average concentrations in air at Wraymires[15] (10^{-9} g kg^{-1})	87	80	26	11	6
Crustal abundance[16] (p.p.m.)	10–15	32–58	37–72	670–1,000	37–72
Soils[17] (%)	1×10^{-3}	5×10^{-3}	2×10^{-3}	6.7×10^{-3}	4×10^{-3}

*Concentrations are taken directly from references and have not been corrected to a simple set of units in as much as their order is of significance.

to the atmosphere[3]. The rate of loss of a substance, W, from a surface is given by

$$W = p\,(M/2\pi RT)^{1/2}$$

where p is the vapour pressure of the substance with molecular weight M, and T and R are the absolute temperature and gas constant respectively. Multiplying W by σ, the land surface area in cm^2 (3.14×10^{18}) and by f, the fraction of the substance in crustal material gives a continental flux. This may be compared with a flux value calculated to give an observed atmospheric burden A in the troposphere, where its tropospheric residence time τ is determined by the removal of the substance with rain. The flux Φ is $\Phi = A/\tau$

then

$$A/\tau = p(M/2\pi RT)^{1/2}\,\sigma f$$

For a substance whose concentration in the atmosphere is C (g cm^{-3}) and the volume of the atmosphere is v, $A = Cv$, but v/σ is the height of the atmosphere h (the average tropospheric height is taken as 13 km). Thus, the equation reduces to

$$p = (h/\tau)[2\pi(RT/M)]^{1/2}(C/f)$$

If τ is taken as ten days, we get

$$p = 3.4\times10^{-2}(T/M)^{1/2}(C/f)$$

Computed values of p may be obtained from the data in Table 1. For Cu, an atmospheric concentration of 50×10^{-15} g cm^{-3}, and a crustal abundance of 50 p.p.m. yields vapour pressures for its species of 6.6×10^{-11} atm at 300 K and 12×10^{-11} atm at 1,000 K. We note that DDT has been dispersed around the Earth primarily in the vapour phase[4], and its vapour pressure is $\sim 2 \times 10^{-10}$ atm (ref. 5) at 300 K. Its mean concentrations in the air over the Sargasso Sea[4] were $\sim 0.42 \times 10^{-16}$ g cm^{-3}.

The role of sublimation in dispersing chemical species around the Earth in the major sedimentary cycle may be significant for such elements as mercury, selenium and arsenic. Weiss *et al.*[6], from rain and atmospheric analyses, suggest that the amount of mercury entering the atmosphere from the degassing of the lithosphere is $\sim 0.25 \times 10^{11}$–1.5×10^{11} g yr^{-1}. The flux of mercury from the continents to the oceans in rivers appears to be much less than that of the continents to the atmosphere. The former flow is estimated to be $\leqslant 3.8\times10^9$ g yr^{-1}. Weiss *et al.*[6] also surveyed man-made mercury pollution. Most probably comes from sulphide mining, which could give a flux of 2×10^9 g yr^{-1}. Thus natural degassing may be a primary path of mercury from the continents to the atmosphere and thence to the oceans.

Atmosphere particulates, collected from the northern, equatorial and southern Atlantic Ocean, southern and eastern China Sea, and various coastal localities, have concentrations (on a gram per gram basis) of Pb, Zn and Sn which are one order of magnitude higher than those in average crustal materials[7], which may be attributed to an anthropogenic source. These three elements could, however, be introduced also by natural degassing, as many of their compounds are volatile.

There is other evidence to support the volatility hypothesis. A large number of Cu and Pb minerals are associated with fumarolic incrustations compared with the number of Mn and Ni minerals[8], and high values of Cu and Pb were, in general, found in atmospheric emissions from Hawaiian volcanoes[9]. Also, anomalously high concentrations of Cu, Pb and Zn were observed in rain which fell in the vicinity of sulphide ore deposits in the Maritime Province[10]. The rain presumably collected these metals from the atmosphere. I note also that rain can introduce halide ions to rock surfaces. The possible formation and subsequent volatilisation of heavy metal halides can enhance rock volatility in as much as such species have higher vapour pressures than their oxides, carbonates and silicates. The relative volatilities of substances have been used to explain the compositions of lunar soils[11].

It therefore would be valuable to determine experimentally the vapour pressures of heavy metals from a suite of common rock types. Such results might lead to more sophisticated field studies to ascertain the role of rock volatility in the major sedimentary cycle and the influence of such processes on the composition of surface seawaters. The upper levels of the mixed layer may receive spikes of volatile chemical species from the continents through rain, whose effect on biological processes could be significant.

The author acknowledges discussions with K. K. Turekian, of Yale University, and J. Hillson and R. McDuff, of the Scripps Institution of Oceanography.

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Received July 11, 1975; accepted January 13, 1976.

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Changes in Canadian national visibility

MAN-MADE pollution can have important effects on public health and the climate[1–3]. The latter, particularly, is a long term effect, and necessitates long term study over wide areas. Here we report the measurement of one relevant

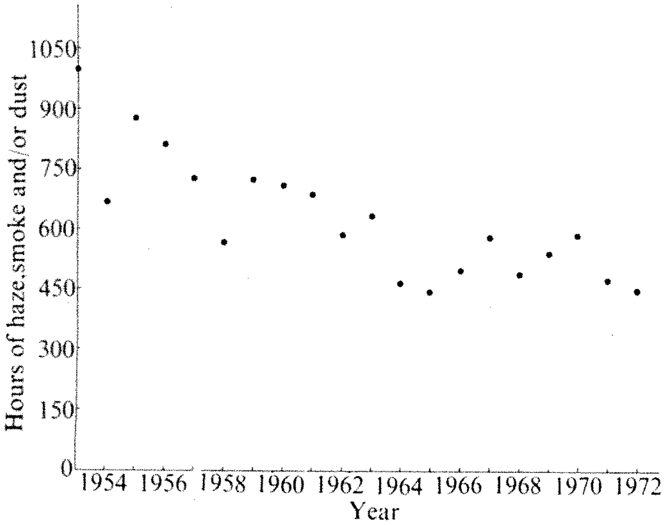


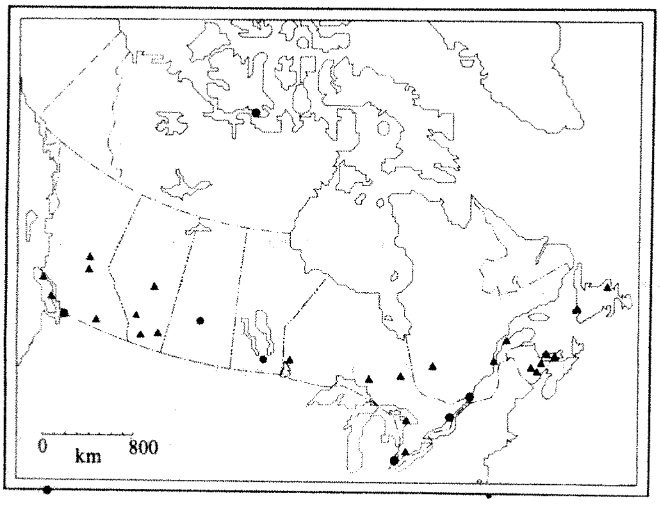
Fig. 1 Weighted average hours of haze, smoke and/or dust per year for major Canadian cities. The data were weighted by the metropolitan populations.

parameter, visibility, at Canadian airports. There are more than 70 usable sets of data. We have found that from 1953 to 1963 there was a general increase in visibility, but, since that date the visibility has remained roughly constant. We attribute both effects to pollution.

Previous work on time trends in visibility and related variables (such as atmospheric turbidity) have generally been limited to a small number of stations and for a short period of time (ref. 4 and other references available on request). Because the data were taken at many different times and places, it is difficult to draw statistically sound judgments on large scale trends. Holzworth (unpublished) has been one of the few to evaluate this quantity on a national basis (for the USA), and determined that visibilities did not deteriorate significantly over a period of ~ 15–20 yr.

Visibility may be affected by a number of pollutants, or change naturally. For example, decreases in visibility have been associated with the presence of ammonia, sulphates, and nitrates[5,7], as well as smoke and other particulates, and models of the relationship of pollutants to visibility have been devised[8]. Visibility measurements themselves have limitations as indicators of pollution levels, because of the effects of humidity, winds and other atmospheric conditions. The difficulties have been carefully discussed by Miller *et al.*[9] and Charlson[10].

Fig. 2 Points with steady increases or decreases in the number of hazy hours for the period 1953–72. The Spearman rank correlation test was used. Others, not showing a steady trend, are excluded. ▲, An increase; ●, a decrease.



There are several ways of evaluating any such trends. To take into account how humans, rather than geographical areas, are affected by changes in visibility, we have weighted the results for each city by its metropolitan population. This was done in computing the points in Fig. 1. The 17 cities included comprise, as of the last census, about half the national population. Other ways of calculating average visibility, for example by dividing observation hours by cumulative miles of visibility (excluding hours in which precipitation occurred), give very similar results to Fig 1. (ref. 11).

Figure 1 indicates that for major Canadian cities, the visibility tended to improve from 1953 to about 1963, when it tended to level off. A pair of lines of regression, both with 1963 as an end point, were calculated. The 1953–63 line had a slope about six times that of the later period.

To find effects on visibility over a wide area, rather than that in cities, we have considered the results of all 71 stations, most of which are not near large cities. For analysis, the Spearman rank correlation method was used, with the annual number of hazy hours compared year by year. Those stations whose change was statistically significant at the 0.05 level are shown in Fig. 2; others are excluded. Large areas, relatively less populated, show a steady increase in hazy days. While some large cities and one northern station have apparently increased their visibility over the two-decade period, the nation as a whole has not shown this trend.

While the visibility at major Canadian cities improved over the first decade of the period considered, there was little or no improvement in the second decade. The explanation for the left-hand portion of Fig. 1 is probably the replacement of coal with less obviously polluting fuels like oil and natural gas; the same phenomenon has been observed in the UK[12] and elsewhere. The post-1963 results are probably caused by a combination of the increased use of all fuels and slight decreases in the amount of pollutants per unit of energy produced. These changes have occurred without national laws on the subject, in contrast to the UK.

The fact that 24 stations of the total of 71 showed a steady increase in haze indicates that visibility outside the metropolitan areas is not improving. This may arise from a worldwide decrease in visibility of an order too small to show up in Fig. 1. The difference between the metropolitan and non-metropolitan trends may explain divergent opinions[13,14] about similar atmospheric data.

The changes described here can induce significant climatic changes. In turn, climatic changes may produce alterations in visibility. By using similar data, we may eventually be able to judge the relationship of world visibility changes to the world climate.

I thank Derek Winstanley for useful suggestions.

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Received July 14, 1975; accepted January 26, 1976.

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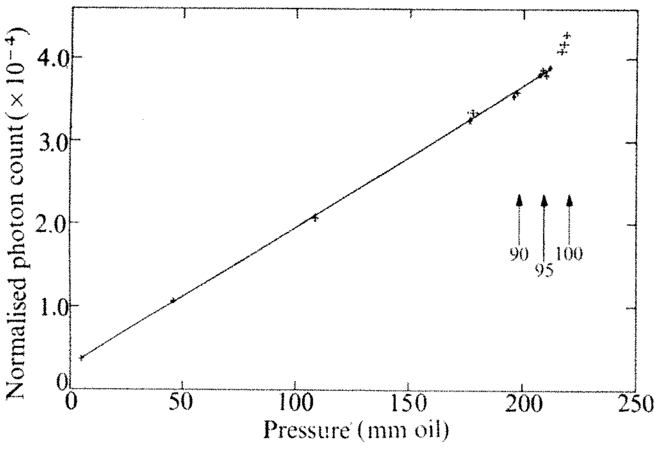
Light-scattering experiments on water vapour at pressures approaching saturation

THE existence of dimers in water vapour at pressures approaching saturation has been postulated for some years and a number of observations have been interpreted in terms of them[1-4]. The relative dimer concentration is expected to increase sharply with increasing percentage saturation and decreasing temperature[1,3]. From speed of sound measurements, a dimer concentration of 8 parts per 10^3 has been reported at 50 °C and 90% saturation[3]; this might be expected to increase to $>20‰$ at 95% saturation and 20 °C. It may be supposed that the polarisability of the water dimer will be considerably larger than that of the monomer, and therefore, using an assumed value for polarisability, an estimate of the dimer concentration at a given temperature and vapour pressure can be made by comparing the quadratic term in a plot of the light scattered as a function of pressure with the known second virial coefficient. In early experiments, using a large multi-pass scattering cell, a very large quadratic term was observed. This arose, however, largely from a rapid increase in the elastic scattering from water-contaminated surfaces as the vapour pressure in the cell approached saturation. We have now eliminated this spurious scattering by selecting spectroscopically only those photons which exhibit a frequency shift due to Brillouin and Raman scattering.

In these experiments two particular difficulties were found: elimination of parasitic light by spectral analysis leads to a large reduction of an already weak signal, and the peculiar problem of reliable pressure measurement with water vapour. Because of surface adsorption, the pressure changes considerably after charging a system with vapour. It was found that long periods, often >1 h, were required before 1% stability could be established in our cell. The consequently long experimental periods thus necessitated very good stability of optics and laser power and constancy of detector response. (For a discussion of high precision photon counting applied to light-scattering studies see ref. 5.)

In our experiments the beam from an argon ion laser (300 mW; 488.0 nm) was directed through a small cell, containing variable amounts of water vapour, and light scattered at 90° to the beam (containing Raman, Brillouin and Rayleigh components) was detected after passing through a confocal, piezo-electrically scanned, Fabry–Perot etalon of free spectral range 375 MHz and finesse ~ 60. The photon counts were registered in a multi-channel analyser. Frequency-shifted light, scattered from the vapour, was selected by counting only photons between Rayleigh peaks. A reference beam, using the same collection system and detector served as a monitor from which small corrections could be made for fluctuations of laser power or detector sensitivity.

Fig. 1 Variation of inelastic scattered light with water vapour pressure at 20.2 °C. A least-squares parabola (indistinguishable from a straight line at this scale) is fitted to the data points up to 95% SVP. Error bars indicate s.d. in photon counts and observed pressure variations. Arrows indicate % SVP.



A representative set of results is shown in Fig. 1 with a least-squares quadratic fit to all data points up to and including the cluster of four observations close to 95% saturation vapour pressure (SVP). The parabola is of the form $S = aP + bP^2$ (where S = photon count and P = pressure) and we find the quadratic term (bP^2/aP) has the value of $2.2 \pm 1 \times 10^{-3}$ at 95% SVP. Values of the second virial coefficient B in water vapour, and its division between a van der Waals' component B_1 and a 'dimer' component B_2, have been given in ref. 4. These suggest that at 95% SVP and 20 °C, $\sim 0.45 \times 10^{-3}$ of the quadratic scattering can be attributed to the increased concentration of monomers because of the van der Waals' attraction (B_1); the remainder $1.75 \pm 1 \times 10^{-3}$ must be attributed to scattering from dimers. The assumption that scattering from a dimer is twice that from the monomer leads to a fractional concentration of dimers of $0.88 \pm 0.5 \times 10^{-3}$—an order of magnitude less than the earlier reported values[3]. On the other hand, the values of B_2 given in ref. 4 would suggest a fractional dimer concentration of only 0.82×10^{-3} at 20 °C and 95% SVP —a value supported by our light-scattering measurements. Alternatively, by adopting this latter value for the concentration, we can calculate the scattering from a dimer as 2.2 times that from a monomer—a figure which is probably quite reasonable in consideration of the likely polarisability of a linear or chain-like dimer[7]. Our experiment consisted of a search for small effects in quasi-elastic scattering, and its accuracy is limited by the peculiar problems of working with water vapour. It seems likely that a Raman scattering experiment should be more definitive, with the possibility of associating specific spectral features with the water dimers.

In conclusion, we note that Fig. 1 shows a number of readings at, and very close to, SVP. In these observations it is likely that significant condensation occurred in the cell, and that small (<0.2 °C) temperature fluctuations during each experiment contributed significantly to small pressure variations. Owing to the possibility of water droplet formation on the windows these results must therefore be interpreted with caution in spite of our monitoring technique. One might, however, expect relatively large concentrations of dimers and multimers at SVP and, as a consequence, the increased scattering which is observed.

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Received August 14, 1975; accepted January 28, 1976.

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Major magmatic activity as a key to predicting large earthquakes along the Sagami Trough, Japan

MIHARA-YAMA is a basaltic stratovolcano on Oshima, an island lying about 20 km off the coast of Izu Peninsula in central Japan (Fig. 1). I attempt here to show that the magmatic activity of Mihara-yama could be used to predict

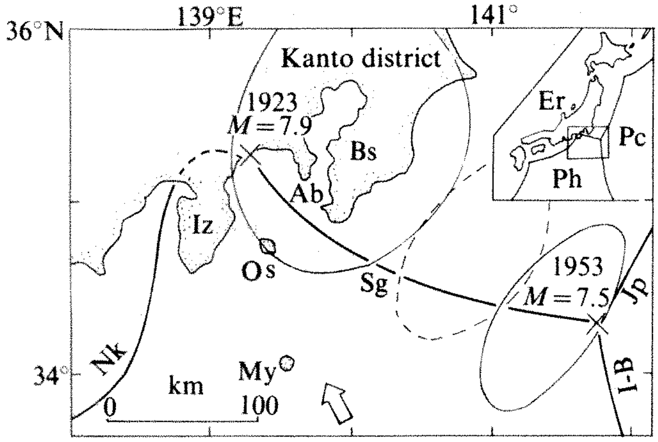

Fig. 1 Large earthquakes that have occurred along the Sagami Trough during this century. The aftershock area is enclosed within the solid line, and the seismicity gap is enclosed within the broken line (see ref. 6). ×, Epicentre of the large earthquake; arrow, direction of slip of the northern Philippine Sea Plate[3]; Sg, Sagami Trough; Nk, Nankai Trough; Jp, Japan Trench; I-B, Izu-Bonin Trench; Os, Oshima; My, Miyakejima; Ab, Aburatsubo; Bs, Boso Peninsula; Iz, Izu Pensinula. Inset: Ph, Philippine Sea Plate; Pc, Pacific Plate; Er, Eurasian Plate.

large earthquakes along the Sagami Trough. There is apparently a causal relationship between eruptions of Mihara-yama and the earthquakes.

The floor of the summit crater of Mihara-yama rose approximately 400 m before the Great Kanto Earthquake of 1923 ($M=7.9$) and the Bosooki Earthquake of 1953 ($M=7.5$), (see ref. 1). Both earthquakes occurred along the Sagami Trough, which extends from the Japan Trench, 180 km south-east of the Boso Peninsula, through Sagami Bay to the city of Odawara on the coast (Fig. 1).

The Sagami Trough forms a border between the Philippine Sea Plate and the Eurasian Plate (ref. 2 and Fig. 1). It is a right-lateral fault with an underthrusting component[3] indicating subduction of the Philippine Sea Plate. Stress between the two plates causes seismic activity along the trough. I have noticed that subsidence of the southern Kanto district around Aburatsubo occurs concurrently with a north-westerly movement of the Philippine Sea Plate. Marked drops in the altitude of the area were recorded before the earthquakes of 1923 and 1953. Further, on both occasions, as the land mass dropped, the floor of Mihara-yama's summit crater rose as much as 400 m, and the volcano erupted.

The earthquakes occurred almost concurrently. The crater's floor fell when the Aburatsubo area rose again after the earthquakes (Fig. 2). It has been suggested that the magma is squeezed up by the compressional crustal strain[1]. I conclude therefore, that the increased compressional crustal stress along the trough forces a downward movement of the Aburatsubo area and squeezes up the magma beneath Mihara-yama, and that, consequently, large earthquakes occur to release strain along the trough. Such activity is regarded as one contributing factor to a major eruption and earthquake.

Mihara-yama's volcanic activity is apparently related to that of Miyakejima, a volcanic island about 80 km south of Oshima. When crustal stress builds up along the trough, Miyakejima begins a cycle of activity of volcanic earthquakes with three frequency maxima, erupting during the second maximum (Fig. 3). Volcanic earthquakes result as stress concentrates beneath the smaller volcano. The stress, though not strong enough to trigger a major eruption of Mihara-yama, does stimulate activity beneath it. In fact, when Miyakejima erupts, a minor eruption occurs on Mihara-yama. When Mihara-yama erupts, all volcanic activity on Miyakejima stops. This implies that strain is first released on Miyakejima, and then accumulates under Mihara-yama. This is a major contributing factor in causing Mihara-yama to enter the active stage. Miyakejima last erupted in 1962. Mihara-yama began its first maximum in 1970; it will probably end sometime in 1975 (Fig. 3).

During the past 200 yr, four large eruptions have occurred on Miyakejima, followed by large eruptions on Mihara-yama at intervals ranging from 2 to 17 yr after activity was first noted on Miyakejima. Large earthquakes followed the eruptions of Mihara-yama within 3–11 yr (ref. 4). Since Miyakejima erupted in 1962, and since Mihara-yama has almost completed its first maximum, we can safely assume

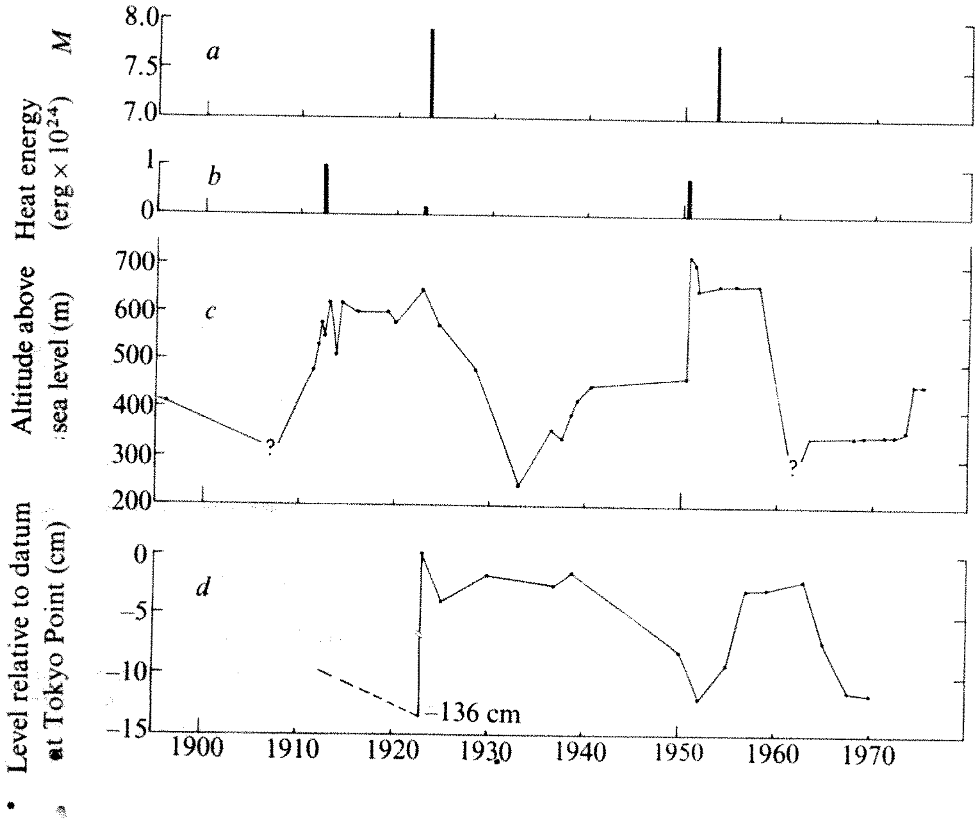

Fig. 2 Relationship between large eruptions, changes in level of floor of summit crater of Mihara-yama, and large earthquakes that have occurred along the Sagami Trough. *a*, Record of large earthquakes along the Sagami Trough (used magnitude data were officially determined by the Japan Meteorological Agency); *b*, heat energy discharged by large eruptions of Mihara-yama[1]; *c*, changes in levels of floor of the summit crater of Mihara-yama (ordinate shows the height above sea level; levelling data to 1958 from refs 7 and 8, after 1958, collected by author); *d*, changes in level at Aburatsubo (solid line measured with precise levelling relative to datum at Tokyo Point[9], and the broken line was deduced from tidal data[10]).

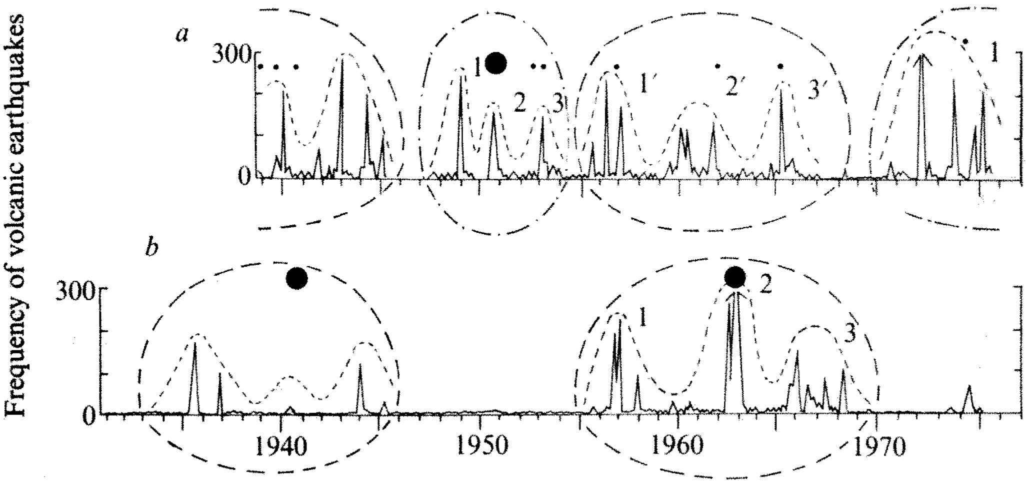

Fig. 3 Relationship between volcanic earthquakes and eruptions in Oshima (*a*) and Miyakejima (*b*) (data from the Japan Meteorological Agency). ●, Eruption points (large dots represent large eruptions; smaller dots represent smaller eruptions which ejected essential magmatic materials). Portions enclosed in the regular broken line, active stages of Miyakejima (*b*). Correlative activity at Oshima (*a*), (relative to datum at Miyakejima) is also enclosed in a regular broken line. Portions enclosed in the irregular broken line (————·————), show active stage of Mihara-yama, Oshima. 1, 2, 3 and 1′, 2′, 3′, maxima of frequency of volcanic earthquakes in respective stages.

that it will erupt sometime between 1975 and 1979. In fact, in 1974 I observed a 100-m elevation of the floor of Mihara-yama's summit crater (ref. 5 and Fig. 2*c*).

This research was partly supported by Kantoken Jishinyochi-chosa Group (K.J.G.).

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Received November 3; accepted December 1, 1975.

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Thermoluminescence of magnesium oxide

MAGNESIUM oxide containing impurity levels of 130–1,400 p.p.m. Ni was investigated for use as a γ-radiation dosimeter, and compared with the widely used LiF (TLD-100). Other samples of MgO containing B, Cr, Fe and Co were also tried but none possesses the required characteristics. It has previously been shown[1] that MgO is suitable for neutron dosimetry when thermally stimulated exo-electrons (TSEE) are used.

The atomic number of MgO is relatively high by comparison with LiF, but this is useful since if the two materials were used in conjunction, it would provide a means of energy discrimination. MgO is also relatively cheap.

Samples of all the MgO types were first exposed to 1 kr. of ^{60}Co γ radiation, and the thermoluminescence (TL) colour observed by eye. A blue colour, suitable for matching the response of the photomultiplier tube in use, was only obtained from Ni-activated samples. Glow curves were then obtained after exposure to 100 r. of the same radiation[2]. The most marked difference between the glow curve of MgO containing Ni and that of pure MgO (ref. 3) and MgO containing other impurities[4], is the presence of a glow peak from 270 °C–320 °C; the peak tends towards the lower temperatures as the exposure is increased. The integrated TL from 75 to 325 °C was obtained for the complete range of nickel impurity concentration and indicated that 370 p.p.m. was the optimum value (Fig. 1). This material was found to have a linear response over the exposure range of 100 r.–100 kr. It was further found that the high temperature peak remained prominent after 14 d at 76 °C, when ~ 40% of the total TL still remained. The The fading of all MgO peaks was considerable (Fig. 2) where samples read out after intervals of 25 h, 9 d and 6 weeks are compared with a LiF TLD-100 extruded chip readout after 6 h. Nevertheless, the previously unreported

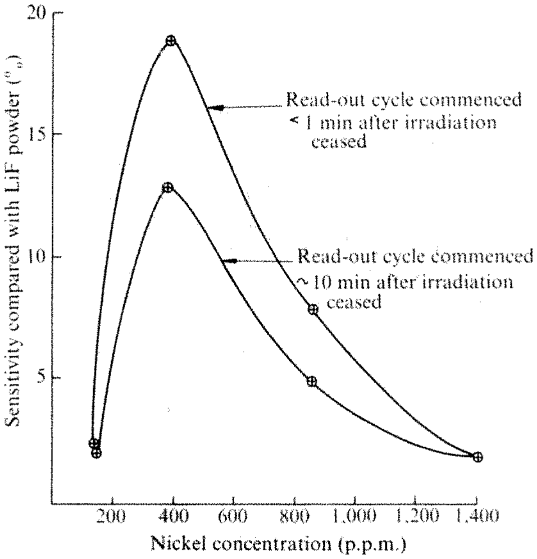
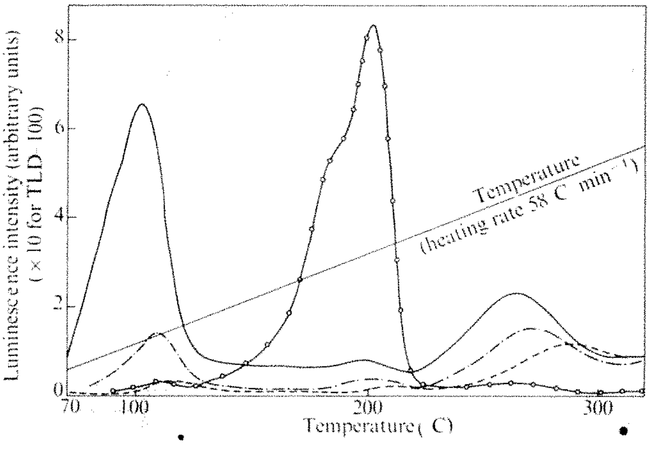


Fig. 1 Variation with concentration of Ni in TL sensitivity of MgO–Ni relative to LiF.

Fig. 2 Glow curves for TLD-100 (chip) and MgO: 370 p.p.m. Ni (chip) exposed to 63 r. ^{60}Co radiation. ——○——○——, LiF after 6 h; ————, MgO after 25 h; —.—.—, MgO after 9 d; – – – – –, MgO after 6 weeks.



high temperature peak in MgO–Ni could be of use for high levels of radiation exposure measurement. The kinetics of the glow curves, energy level values and luminescence spectra are being investigated.

I thank Dr W. B. Gilboy (University of Surrey) for supervising this work.

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Received July 7, 1975; accepted January 28, 1976.

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Influence of magnesium on nickel toxicity

THE influence of calcium in ameliorating heavy metal toxicity towards plants is well known. The possibility that magnesium might possess similar properties, if to a lesser extent, has been largely ignored by ecologists although there are indications in the literature that such is the case[1,2]. We here reaffirm the effect, and point out its ecological significance.

We investigated the growth of oats (*Avena sativa* L. var. Selma) over a wide range of nickel and magnesium concentrations in water culture. We here report in detail one of our experiments in which oats were grown at magnesium levels of 1.25, 2.5, 5.0 and 7.5 mM combined with nickel at 0 and 1.5 p.p.m. The oats were pregerminated and transferred as seedlings to containers filled with 1.6 l of culture solutions. The culture solutions contained, in addition to the experimentally varied levels of $MgSO_4$ and $Ni(NO_3)_2$, 1 mM $NH_4H_2PO_4$, 6 mM KNO_3, 0.5 mM $Ca(NO_3)_2$, 7 mM $NaNO_3$, 0.1 mM NaCl, and, as micronutrients, 90 µM NaFeEDTA, 46 µM HBO_3, 9.1 µM $MnSO_4$, 0.76 µM $ZnSO_4$, 0.32 µM $CuSO_4$, 9.1 µM $(NH_4)_6Mo_7O_{24}$. The *p*H was adjusted to 5.2 at each weekly change of culture solution.

The experimental layout was of a randomised block design; there were 64 containers in all, each with three oat plants, with 8 blocks for each treatment. The experiment was run for 35 d in a shaded glasshouse with 18 h d^{-1} supplementary light. Individuals were then scored for Ni toxicity symptoms and harvested. They were dried at 75 °C, weighed on a per pot basis, and the results (Table 1) subjected to an analysis of variance which indicated significant main effects of both Ni ($P < 0.001$), Mg ($P < 0.05$) and Ni–Mg interaction ($P < 0.025$).

Figure 1 shows that the characteristic Ni toxicity symptoms (described in detail by Vergnano and Hunter[3]) decreased in severity with increasing Mg concentrations. The dry weight measurements (Table 1) showed no significant difference between the oats grown at 0 p.p.m. Ni and 1.25, 2.5 and 5.0 mM Mg. The fall in yield at the 7.5 mM level is presumed to result from an excess of Mg. In contrast, there were marked increases in weight over the range 1.25–5.0 mM Mg at 1.5 p.p.m. Ni. This experiment, and a number of others not reported in detail here, show that Ni toxicity can be ameliorated by increasing Mg levels. (We have no evidence that sulphate anions influence Ni toxicity over the concentration ranges used.)

Table 1 Mean dry weight ± s.e. (g) of oat plants grown in culture solutions with differing magnesium and nickel levels. We have, for brevity, combined root and shoot data: their response is similar, although the Ni–Mg interaction is more marked for the roots

Mg (mM)	Ni (p.p.m.) 0	1.5
1.25	2.01±0.19*	0.83±0.17
2.50	1.99±0.17	1.21±0.17
5.00	2.02±0.17	1.62±0.17
7.50	1.38±0.17	1.35±0.17

*Mean of seven observations only.

A further aspect of the influence of Mg on Ni toxicity was shown in an experiment using very high levels of magnesium (25 and 40 mM) with high levels of nickel (5 and 10 p.p.m.). At 40 mM magnesium the plants were very badly stunted, but showed no symptoms of the toxicity of Ni, and were indistinguishable from plants subjected to the same high level of Mg with no Ni. At 25 mM Mg, the toxicity symptoms of Ni were completely suppressed at the 5 p.p.m. Ni level, but were visible to a small extent at the 10 p.p.m. level.

Further work is in progress to investigate the means by which Mg ameliorates Ni toxicity, but it seems worthwhile to comment on the ecological significance of these preliminary observations. Serpentine soils very often contain high levels of Mg and relatively large quantities of Ni, and many authors have used oats as a bioassay for the presence of toxic levels of Ni in these soils[4,5]. The failure to detect Ni toxicity in a number of such soils (ref. 4 and J.P., unpublished) in spite of evidence that appreciable quantities of Ni were present, may arise partly from the influence of high levels of Mg. The use of oats as a bioassay for Ni toxicity can now be seen to involve an oversimplification of the soil situation and assumes that the Ni–Mg interaction is similar for other species.

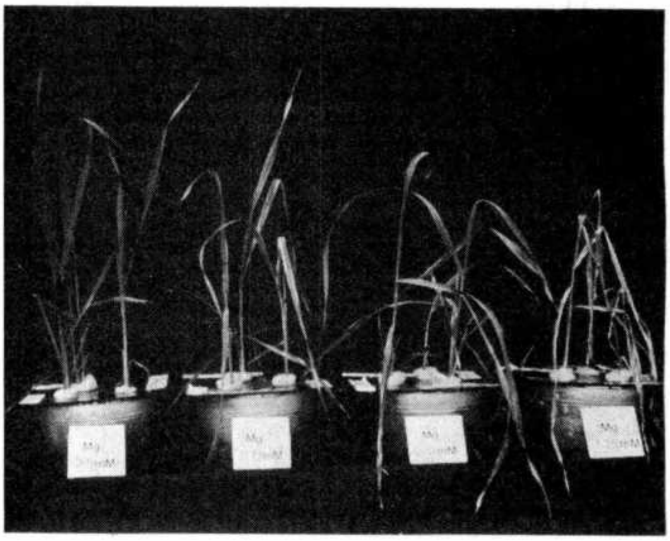

Fig. 1 Increasing symptoms of nickel toxicity in oats with decreasing levels of magnesium (from left to right: 7.5; 5.0; 2.5; and 1.25 mM).

The response to Mg of other Ni-sensitive species remains to be investigated, but we feel a consideration of Ni–Mg interactions must be involved in future studies on serpentine soils.

We thank Mr D. Machin for statistical help, and the staff at the glasshouses of the University of Stirling for their help in maintaining the water cultures.

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Received November 24; accepted December 12, 1975.

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Interoceanic differences in vulnerability of shelled prey to crab predation

SHELLED gastropods living on intertidal open rocky surfaces exhibit various shell features which can be interpreted as adaptations against such shell-breaking predators as fishes, spiny lobsters, and crabs. These anti-predatory devices include strong external shell sculpture, elongate or dentate apertures and low spires[1]. Not only are these characteristics more common among tropical species compared with ecologically similar cold-water forms, but within the tropics they are more prevalent and better developed in the Pacific and Indian Oceans than in the Atlantic[1,2]. Any or all of three conditions must obtain in areas with a high incidence and strong development of anti-predatory armour compared with areas where such armour is reduced: the prey are stronger, that is, the criteria needed by the prey to avoid being crushed are more stringent; the predators are stronger; and shell-breaking predators account for relatively greater mortality. Here I report data confirming the first two of these possibilities.

At the University of Guam Marine Laboratory (Pago Bay, Guam, Mariana Islands), various reef-flat crabs (Table 1) were found to feed readily on shelled gastropods and hermit crabs by crushing them in their massive master claw. Individual crabs were kept in aquaria with aerated or recirculating seawater. The critical size (maximum size of vulnerability) of prey was obtained for individual crabs by offering them a range of sizes of that prey species. The common occurrence in the field of snails whose shells show repair after presumed damage by a predator suggests that shell breakers often attack prey that are too large for them.

Direct comparisons between Guamanian and ecologically similar West Indian prey were made by allowing hermit crabs to inhabit Jamaican shells, which were then offered to the predatory crabs. Shells were collected alive near Discovery Bay, Jamaica; the soft parts were removed and the shells dried in air before being used in Guam 4–9 months later. Shells treated in this way seem to possess mechanical properties indistinguishable from those of living snails[3].

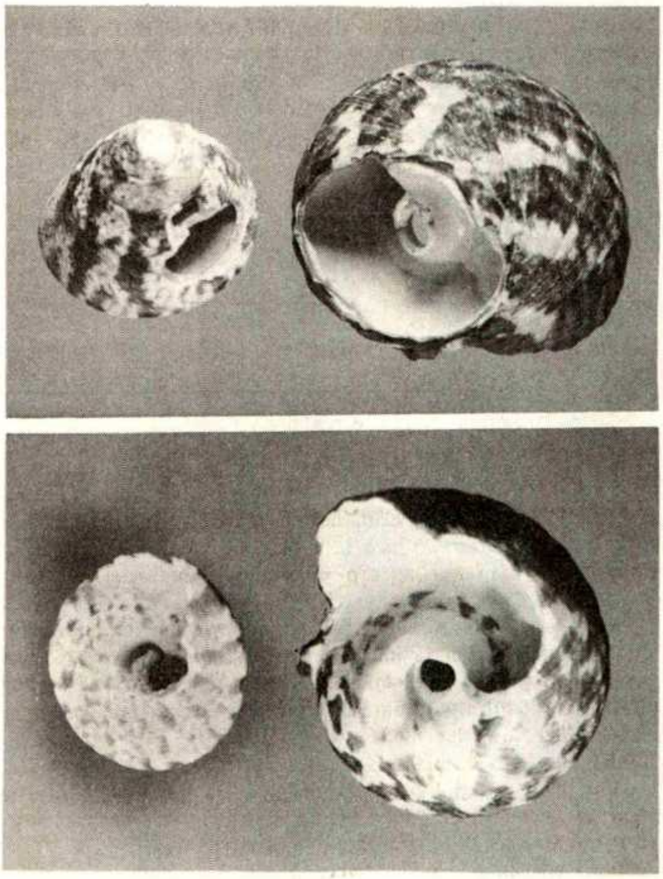

Fig. 1 Shells of the Jamaican *Cittarium pica* (left) and Guamanian *Trochus niloticus* (right) broken by the Pacific crab *Carpilius maculatus*. The two views of each species pertain to the same shell. Photograph by F. Dixon.

Although in most cases not enough shells were at hand to establish the critical size of Jamaican prey, it is evident from Table 1 and Fig. 1 that this critical size is greater than that of ecologically equivalent and taxonomically related Guamanian snails. Since the largest offered specimens of all West Indian species were extensively damaged, it is likely that the critical

Table 1 Critical sizes and shell thicknesses of Guamanian (G) and Jamaican (J) shelled prey crushed by Guamanian predatory crabs

Crab Species	Crab Width (mm)	Prey Species	Prey Critical size (mm)	Largest size offered (mm)	Shell thickness (mm)
Carpilius maculatus	115.3	(G) *Trochus niloticus*	39.5	49.0	1.3–2.5
		(J) *Cittarium pica*	57.4	57.4	1.1–3.8
	138.1	(G) *T. niloticus*	39.5	45.1	—
		(J) *C. pica*	55.3	55.3	—
Carpilius convexus	89.9	(G) *T. noliticus*	37.0	38.2	—
		(J) *C. pica*	48.2	48.2	—
Daldorfia horrida	82.8	(G) *T. niloticus*	35.0	42.0	—
		(J) *C. pica*	38.0	38.0	—
Eriphia sebana	65.3	(G) *T. niloticus*	25.3	31.1	—
		(J) *C. pica*	34.0	34.0	—
Carpilius maculatus	115.3	(G) *Conus spp.*	30.5	37.2	1.4–2.8
		(J) *Conus mus*	39.7	39.7*	1.1
Daldorfia horrida	82.8	(G) *Conus spp.*	31.3	40.3	—
		(J) *Conus mus*	32.5	32.5	—
Eriphia sebana	56.1	(G) *Nerita albicilla*	23.6	24.3	1.1–1.9
		(J) *N. tessellata*	20.9	20.9*	0.8–1.5
	65.3	(G) *N. albicilla*	27.1	30.0*	—
		(J) *N. tessellata*	20.7	20.7*	—
	56.1	(G) *N. plicata*	21.0	22.6	1.3–1.7
		(J) *N. versicolor*	21.7	21.7	0.9–1.0
		(J) *N. peloronta*	30.0	30.0	0.8–1.0
	65.3	(G) *N. plicata*	27.9	27.9*	—
		(J) *N. versicolor*	27.2	27.2*	—
		(J) *N. peloronta*	34.5	34.5	—

*Close to, or equal to, maximum adult size.

Critical sizes for Guamanian prey are defined as the mean between the largest specimen crushed and the next larger one which remained intact or which sustained only minor damage to the outer lip. These two sizes were never more than 1.5 mm apart.

Table 2 Vulnerability of *N. lapillus* to predation by Guamanian (G) and Irish (I)[3,4] predatory crabs

Crab species	Width (mm)	Thick high-spired form Largest crushed (mm)	Thick high-spired form Largest given (mm)	Thin low-spired form Largest crushed (mm)	Thin low-spired form Largest given (mm)
(G) *Carpilius maculatus*	105.4	29.0	29.0	—	—
	111.3	29.4	29.4	—	—
	115.3	33.4	33.4*	—	—
(G) *Carpilius convexus*	89.9	27.2	27.2	—	—
(G) *Daldorfia horrida*	82.8	32.8	32.8	—	—
(G) *Eriphia sebana*	56.1	27.5	27.5	—	—
(I) *Portunus puber*	80	20	—	25	—
(I) *Carcinus maenas*	59	22	24	24	27
	66	12	27	25	27
	75	12	27	25	29

*Close to maximum adult size in Maine.

sizes of these species are markedly larger than the maximum sizes available in my experiments. Even so, the largest successfully crushed *Cittarium pica* L. are about 1.5 times as large in body-whorl diameter as the Guamanian analogue, *Trochus niloticus* L. The superiority of *Trochus* over *Cittarium* stems in part from its relatively smaller umbilicus and proportionately smaller extent of outer shell wall not supported from within by earlier whorls (Fig. 1). *T. niloticus* also attains a larger adult size (150 mm compared with 110 mm in *C. pica*).

Table 3 Relative manus height and manus thickness in the Indo–West-Pacific (P) and Western Atlantic (A) species of *Carpilius* and *Eriphia**

Species	Carapace width (mm)	Manus height / Carapace width Mean	Manus height / Carapace width Range	Manus thickness / Carapace width Mean	Manus thickness / Carapace width Range	*n*
(P) *Carpilius maculatus* L.	63.6–138.1	0.422	0.345–0.495	0.245	0.223–0.260	14
(P) *C. convexus* Forskal	56.1–90.1	0.386	0.358–0.424	0.234	0.211–0.261	15
(A) *C. corallinus* Herbst	61.3–142.3	0.355	0.327–0.406	0.211	0.202–0.247	11
(P) *Eriphia sebana* Shaw and Nodder	36.7–65.3	0.503	0.411–0.605	0.314	0.262–0.366	23
(P) *E. smithii* McLeay	34.9–65.1	0.475	0.418–0.504	0.315	0.269–0.371	8
(P) *E. scabricula* Dana	21.0–24.4	0.440	0.414–0.463	0.308	0.299–0.314	4
(A) *E. gonagra* Fabricius	18.8–43.7	0.416	0.373–0.485	0.288	0.254–0.344	17

*All intrageneric differences are significant at the 0.01 level or better (Mann–Whitney *U* test) except that for manus thickness between *E. sebana* and *E. smithii*. *E. scrabricula* was not statistically compared with other species, and is included only for completeness.

The Jamaican *Conus mus* Hwass is thinner-walled (Table 1), higher-spired, and more broadly apertured than any Guamanian *Conus* normally found on hard substrata (*C. chaldaeus* Röding, *C. coronatus* Gmelin, *C. ebraeus* L., *C. flavidus* Lamarck, *C. lividus* Hwass, *C. miles* L., *C. sponsalis* Hwass). Among high intertidal species of *Nerita*, the West Indian *N. peloronta* L. and *N. versicolor* Gmelin are thinner-shelled and more vulnerable to predation by the intertidal crab *Eriphia sebana* than the Guamanian *N. plicata* L. (Table 1). The lower intertidal *N. tessellata* Gmelin from Jamaica is smaller and more vulnerable than, but comparable in shell thickness with, its Guamanian analogue *N. albicilla* L. (Table 1)

In other trials, individual crabs were offered hermited specimens of the cold-temperate snail *Nucella lapillus* L., whose North Atlantic crab predators (*Portunus puber* L., *Carnicus maenas* L. and *Cancer pagurus* L.) have been well studied in Ireland[4,5]. Thick-shelled high-spired specimens of *Nucella* were collected in August 1971, from sheltered sites near Boothbay Harbor, Maine. Table 2 suggests that Guamanian crabs can crush larger *Nucella* and are more powerful than similarly-sized Irish crabs.

Among the factors affecting the strength of a crab's master claw is the cross-sectional area of muscle in the manus (that part of the propodus behind the apposing fingers). Both components of cross-sectional area, manus height (dorsiventral dimension) and manus thickness (anteroposterior dimension), were measured on crabs in the US National Museum, Washington, DC. Within the genera *Eriphia* and *Carpilius*, both components of cross-sectional area relative to carapace width are greater among Indo–West-Pacific taxa than in their Caribbean counterparts (Table 3). No differences in manus height or manus thickness could be detected between males and females of either *Carpilius maculatus* or *C. convexus*, but in the other species (Table 3) the manus of the males is higher and thicker than that of the females. Differences between species are preserved even when only females or only males are compared. I found no differences in claw form between different populations of the same species, and there seems to be no allometry of either manus height or manus thickness with increasing carapace width.

In summary, the arms race between Indo–West-Pacific rocky-shore gastropods and their crab predators has progressed further or remains in a more escalated state than in other tropical or temperate regions. Presumably, this high degree of coadaptation is related in part to the great age and stability of the tropical Pacific and Indian Oceans. The applicability of this finding to gastropods in other habitats, and to shell-breaking predators other than the xanthid and parthenopid crabs considered here, remains to be determined.

I thank M. Yamaguchi for allowing me to use his laboratory during the summer of 1975, and E. Zipser and E. Dudley for assistance. Supported in part by the Oceanography Section, National Science Foundation, and by the John Simon Guggenheim Memorial Foundation.

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Mechanism for digestion of detrital bacteria by the cichlid fish *Sarotherodon mossambicus* (Peters)

ALTHOUGH many species of fish ingest large amounts of detritus, its nutritional value has been questioned frequently[1,2]. Detritus in marine and freshwater habitats consists of a mixture of plant debris and amorphous organic matter together with associated heterotrophic and autotrophic microorganisms[3]. In detritus which does not contain large amounts of undegraded vascular plant matter, most of the protein is associated with heterotrophic microorganisms[5–8]. This is particularly true in fresh water[9]. Thus, the value of detritus as a food for fish depends to a large extent on their ability to digest heterotrophic microorganisms. It has been suggested that fish which consume detritus may have this ability[4]. Using ^{14}C-labelled bacteria in *in vitro* experiments I have now demonstrated it in *Sarotherodon mossambicus*.

Rapidly growing juvenile *S. mossambicus* (2.0–8.0 cm standard length) in Lake Sibaya (32°40′E, 27°25′S), KwaZulu, feed entirely on benthic detritus which contains large numbers of diatoms, bacteria and amorphous organic material (my unpublished work). Gastric *p*H in specimens examined immediately after capture was measured with test papers (Acilit, Merck) read to 0.25 *p*H units. In stomachs of actively feeding fish, *p*H was consistently below 2.5, with values of 1.5 and 1.25 most common, and occasionally a *p*H of 1.0 was measured. Intestinal *p*H ranges from 8.0 to 8.8 (ref. 10). Residence time of material in the stomach is estimated to be about 1 h. Although gastric *p*H values below 2.0 are very unusual in vertebrates, Moriarty[11] found that the cichlid fish *Tilapia nilotica* in Lake George, Uganda, uses gastric acid below *p*H 2.0 to lyse blue-green algae. Because of the chemical similarity between bacterial and blue-green algal cell walls, it seemed likely that the unusually low gastric *p*H found in *S. mossambicus* would cause lysis of bacteria.

The effect of different gastric *p*H values on the extent of subsequent intestinal digestion of bacteria was investigated in *in vitro* simulations of digestion in *S. mossambicus*. Pooled stomach and pooled intestinal contents of ten actively feeding *S. mossambicus* were centrifuged and the supernatants decanted and stored at −10 °C until required. Protease activity in stomach juice was determined with a sensitivity of 2 μg pepsin per ml by the method of Anson and Mirsky[12]. Detritus was collected from shallow sand terraces in Lake Sibaya, and bacteria in 20 ml of suspended detritus were labelled differentially with ^{14}C-glucose (271 mCi mmol^{-1}) for 1 h at a concentration of 32.4 μg glucose per l. It has been shown that concentrations of glucose below 500 μg l^{-1} are taken up by bacteria but are not high enough to be utilised by algae[13]. Unassimilated ^{14}C label was removed by repeated suspension of the detritus sample in sterile water followed by centrifugation. The detritus was then resuspended in 20 ml of sterile water, and 3 ml of suspended detritus was transferred to each of six centrifuge tubes.

To simulate stomach conditions, hydrochloric acid was added to the detritus in five of the six centrifuge tubes to produce a range of *p*H values from 1.3 to 2.5. To compare acid and enzymatic lysis, lysozyme (2 mg in 2 ml of phosphate buffer, *p*H 6.2, from egg white, Sigma) was added to detritus in the sixth centrifuge tube. The tubes were then stoppered and placed horizontally in a shaking water bath at 25 °C. After 1 h, the six suspensions were centrifuged and the levels of radioactivity in 0.1 ml of each of the supernatants was determined by liquid scintillation counting. The remaining supernatant was discarded.

To simulate intestinal digestion, each detrital pellet was resuspended in 3 ml of intestinal juice at *p*H 8.6. Boiled intestinal juice was used as a control. Release of ^{14}C from bacteria was monitored by centrifuging the suspensions periodically and determining radioactivity in 0.1 ml of each of the supernatants. After sampling, the pellets were resuspended quickly and the tubes were returned to the water bath.

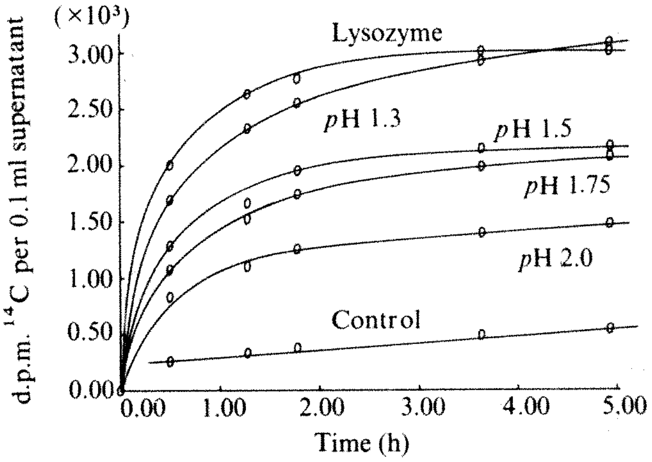

Fig. 1 Radioactivity released from bacteria during treatment with intestinal juice after acid treatment at *p*H values shown. Data given are experimental minus control with correction for volume reduction caused by sampling.

No label was released from bacteria during treatment with HCl, but after 30 min subsequent treatment with intestinal juice, large amounts of label were released relative to the control. Label continued to be released for up to 3.5 h, but no significant additional release was found thereafter. The total amount of label released was indirectly proportional to *p*H during treatment with HCl such that the activity released from bacteria treated at *p*H 1.3 was twice that released from bacteria treated at *p*H 2.0 (Fig. 1). The amount of label released from bacteria treated at *p*H 1.3 was similar to that released from bacteria treated with lysozyme. These results show that exposure to low *p*H lyses bacteria with an efficiency comparable with that of lysozyme and thus makes bacterial cell contents available to digestive enzymes in the intestine.

Gastric *p*H conditions required for bacterial lyses would be expected to be unfavourable for gastric enzymes. The normal *p*H of actively digesting vertebrate stomachs is approximately 2.0 and gastric enzymes usually show optimal activity at *p*H 2 or above. Fish[10] and Nagase[12] report *p*H optima of 2.2 and 2.8 respectively for protease from the gastric mucosa of *S. mossambicus*, with greatly reduced activity at lower *p*H. Pooled stomach juices from the ten *S. mossambicus* used in this experiment had a *p*H of 1.75 and no protease activity was detected. Moriarty[9] found a similar lack of enzyme activity in the stomach of *Tilapia nilotica* which use gastric acid to lyse blue-green algae. These results suggest that gastric enzymes are not secreted when gastric *p*H would result in enzyme activities well below optimum.

Of the four important groups of fish which feed on detritus; *Chanos chanos*, mullets, carps and tilapia (including *S. mossambicus*), only tilapia have true stomachs with gastric glands[14]. Thus, the mechanism for lysis of detrital bacteria reported here is probably unique to the tilapia, and other mechanisms may await discovery in the other groups.

I thank Professors B. R. Allanson, and J. C. van Hille, and Drs J. R. Moss and I. Railton for critically reviewing the manuscript and Mr S. Robertson for the protease assay.

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Received November 28, 1975; accepted January 30, 1976.

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Mescaline as a mitotic spindle inhibitor

COLCHICINE and its methyl derivative colcemid not only inhibit microtubule self-assembly[1] but also affect fast transport in nerve[2]. A report that mescaline likewise inhibits the rapid component of axoplasmic transport in nerve[3], led us to investigate the effects of this drug on mitosis, and on purified microtubule protein (tubulin[4]). The structure of mescaline does resemble an abbreviated version of colchicine (Fig. 1). We report here that mescaline can, like colcemid, be used as a mitotic spindle inhibitor and has certain advantages as a cytogenetic reagent.

We first investigated the effect of a range of concentrations of mescaline from just above to just below the limits at which concentrations of colcemid exert an inhibitory influence on mitosis. When human skin fibroblasts are cultured in small dishes, the dividing cells round up so that by mid-metaphase they stand out in marked contrast to the flat cells with a polygonal outline characteristic throughout interphase. If the rounded cells are examined using Nomarski interference optics[5], they can be seen to be in division as one can distinguish the chromosomes in the live cell (Fig. 2). By simply counting the number of rounded cells in a dish, under an ordinary dissecting microscope, one can score any compound for its effect as a mitotic inhibitor.

This first series of experiments (Fig. 3) showed that mescaline was similar to colcemid in its effectiveness. At the higher concentrations, cells treated with colcemid showed some signs of a toxic effect (detachment); those treated with mescaline did not.

A second series of experiments (Fig. 4) designed to study the time course of events up to 24 h after the inoculation of fibroblast cultures with mitotic inhibitors, showed that mescaline is metabolised rather readily, giving a peak of inhibition at 4 h followed by a plateau of moderate inhibition. The first breakdown product of mescaline in human brain is *N*-acetylmescaline[6]. We synthesised this compound, and found that it acts in our experimental system as a moderate inhibitor of mitosis. We therefore attribute this plateau effect to the metabolite *N*-acetylmescaline. Colcemid was also metabolised, but more slowly. It is likely that the first step in this process is the demethylation of a methoxy group in ring A of the molecule, as reported for colchicine in mammalian liver[7,8]. Results with 1,4-dichlorobenzene, which is known to be an effective inhibitor of mitosis in plants[9], gave no indication that this agent is metabolised at all.

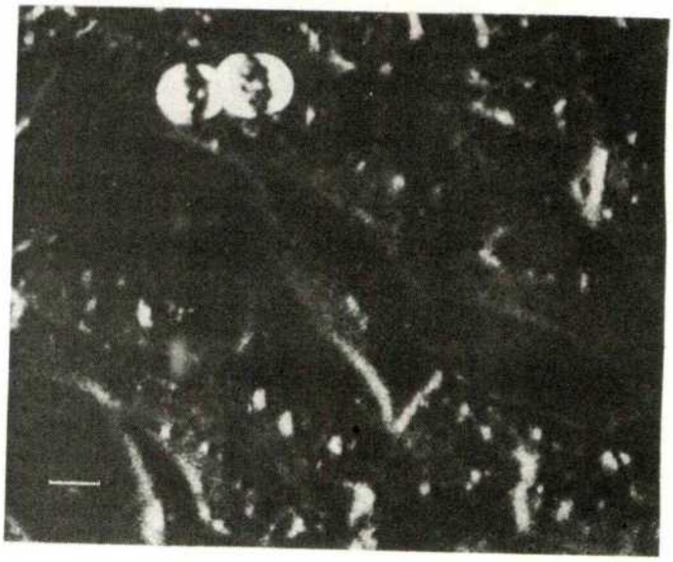

Fig. 2 Nomarski interference image of cells in division. Cells at the top left are in anaphase, with the chromosomes visible on the spindle. The rounded appearance of the dividing cells is in marked contrast with the polygonal interphase cells seen below. The micrograph shows the progress of division past metaphase in the absence of any drug treatment. Bar indicates 20 μm.

Our conclusion is that mescaline could be used as a mitotic inhibitor for use in cytogenetic work. Our observations with a variety of human and other mammalian cells show that this drug produces well-spread metaphases suitable for examination by banding techniques (Fig. 5). Mescaline has the advantage that it is 1,000 times less expensive to purchase than is colcemid; it has the disadvantage that its use is controlled by drug legislation.

Our experiments with radioactive mescaline demonstrated that it binds to purified microtubule protein. Like colchicine, it also inhibits the assembly of tubulin subunits to form microtubules. The implications of these findings should be of some general interest. Mescaline is a catecholamine-like neurotransmitter analogue. Its effects on tubulin raise the possibility that neurotransmitters or their

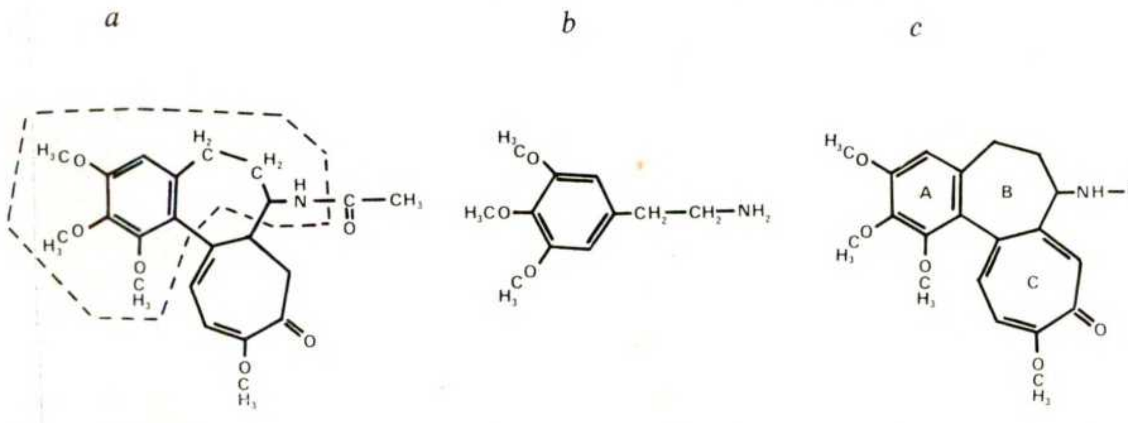

Fig. 1 Structure of spindle inhibitors. *a*, Colchicine; *b*, mescaline; *c*, colcemid. Dotted line indicates the portion of the colchicine molecule to which mescaline would seem to correspond.

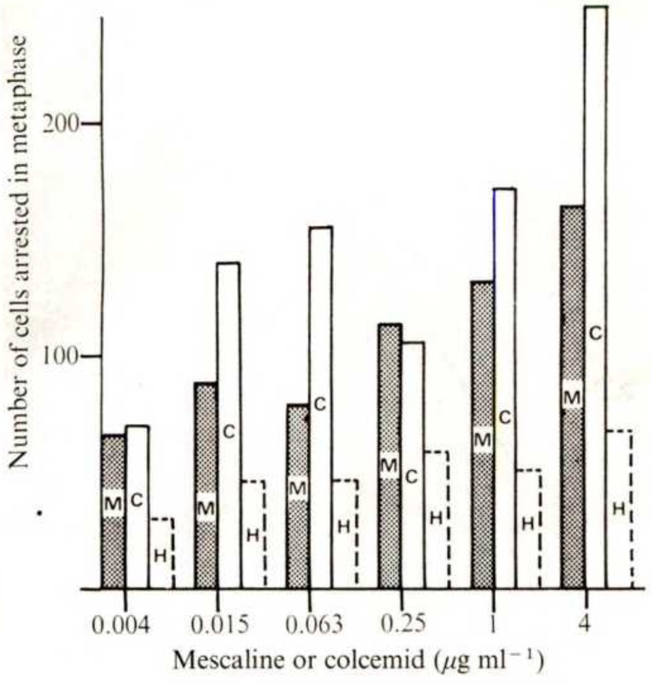

Fig. 3 Comparison of dose responses of human skin fibroblasts to mescaline and colcemid. Cells (2×10^4 per 3-cm Petri dish) were plated out, fed with fresh medium the following day, and exposed to the spindle inhibitors at the dosages indicated. Cell counts were begun 2 h after the start of the treatment and are expressed as the average of a forward and a backward count through the series of dishes. Slight effects of cooling and disturbance are reflected by minor increases in counts in the control dishes. Cells rounding up due to toxic effects at high dosage of colcemid may make some contribution to the count at 4 μg colcemid ml^{-1}. M, Dishes given mescaline in 0.1 ml Hanks' balanced saline; C, dishes given colcemid in 0.1 ml Hanks' balanced saline; H, control dishes given 0.1 ml Hanks' balanced saline.

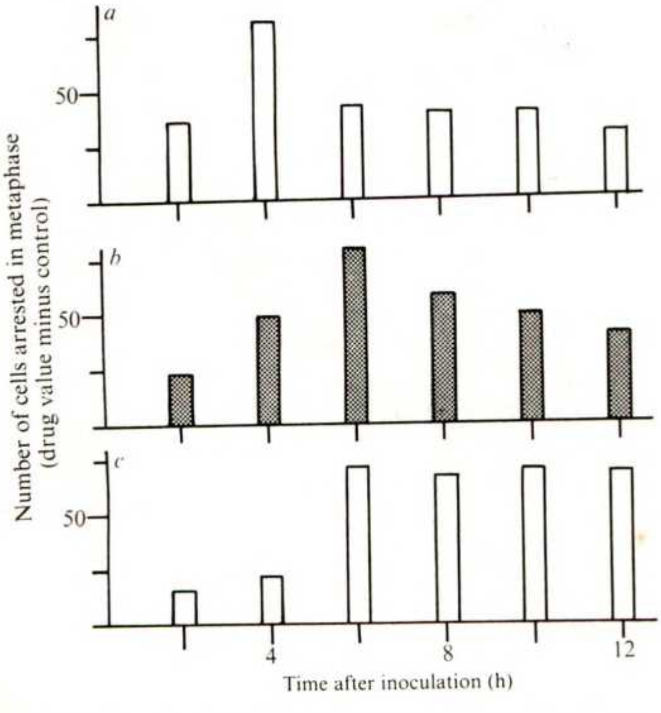

Fig. 4 Time course of effects of spindle inhibitors on human skin fibroblasts. Cells were plated out as in Fig. 3. Counts were made once on each of six sets of dishes at 2 h intervals, each set included a control inoculated with Hank's balanced saline, a dish with colcemid (*a*) at 4 μg ml^{-1}, a dish with mescaline (*b*) at 5 μg ml^{-1}, and a dish inoculated with 0.1 ml saturated solution (*c*) of 1,4–dichlorobenzene in Hanks' balanced saline.

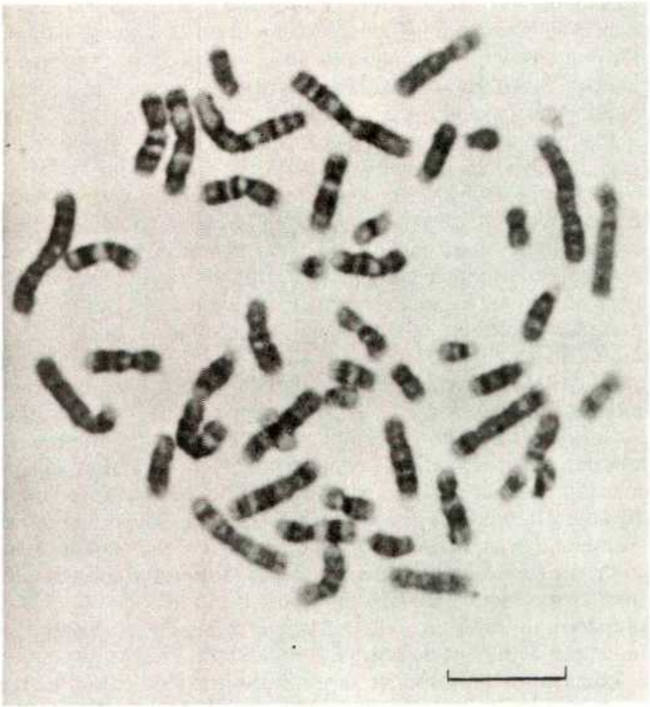

Fig. 5 Metaphase spread from a human leukocyte culture prepared with mescaline as a spindle inhibitor, trypsin-banded and stained with Giemsa. Bar indicates 10 μm.

metabolites may have a separate role additional to their effect at the cell membrane—regulation of the polymerisation state of fibrous proteins within the cell. The biochemical results of this work will be reported separately.

We thank the MRC for supporting part of this work. This work was carried out under Home Office licence.

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Received September 3, 1975; accepted February 4, 1976.

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Antibodies to a cell-surface component coded by human chromosome 21 inhibit action of interferon

ANTICELLULAR sera able to block specific cell responses are important in the study of surface receptors. Particularly useful are antibodies against the human cellular antigens coded for by a specific chromosome, which can be prepared by immunising mice with mouse–human somatic cell hybrids containing defined human chromosomes[1]. We have applied this method to the study of the mechanism by which the presence of human chromosome 21 renders such hybrids sensitive to human interferon[2]. Interaction of an interferon with sensitive cells induces intracellular biochemical changes, including the formation of an inhibitor of protein synthesis, making the cell unable to support virus replication[3,4]. We have obtained antibodies to hybrid cells containing human chromosome 21, and these inhibit the response of live human cells to interferon. Our experiments indicate that chromosome 21 codes for a cell-surface component required as a specific receptor for human interferon.

For immunisation, C3H/FcJ mice were inoculated intraperitoneally once a week with 10^7 cells from clone WAVR4d (ref. 5) originating from the fusion of a mouse A9 cell and a human diploid fibroblast WI-38: this clone contains human chromosomes 4, 21 and 22 only. After 4 weeks, immune serum was collected and decomplemented at 56 °C for 30 min. The titre of antibodies was estimated by cytotoxicity tests[6] in the presence of rabbit complement. At a dilution of 1/128 the killing index was 70% on WAVR4d and 20% on mouse A9 cells. At 1/32, they were 90 and 70, respectively. The response to mouse cell was lowest for short periods of immunisation. Adsorption of the antiserum on A9 cells eliminated the mouse-specific reaction, while keeping intact the reaction against hybrid cells. No reaction was seen with hybrids whose human chromosomes do not include 4, 21 or 22. Reaction was observed with HeLa cells. Most important, no cytotoxicity was observed in the absence of complement, which is the condition used in the interferon assay. Human interferon (5×10^4 U ml^{-1}) was prepared in foreskin cells FS4 (given by Dr J. Vilcek) by induction with poly(rI)·poly(rC)[7].

Effects of anti-WAVR4d serum were first studied on the line of hybrid cells used for immunisation (Table 1). These cells contain human chromosome 21 and are therefore sensitive to human interferon (although they require at least 10 times more interferon than normal human cells). Hybrids lacking chromosome 21 do not respond to human interferon. Addition of the antiserum completely inhibits the effects of human interferon on vesicular stomatitis virus (VSV) replication in the sensitive hybrid cells. To establish that the inhibition of interferon's action is due to reaction with human immunodeterminants, we next examined the effect of the antihybrid serum on normal human diploid fibroblasts.

Pretreatment of human foreskin cells FS7, with anti-WAVR4d serum inhibited the antiviral effect of interferon (Fig. 1). At low interferon concentrations, the inhibition of VSV replication is completely abolished after exposure to the antibodies. Excess of interferon can overcome the effects of antiserum pretreatment. At intermediate interferon concentrations, more than 100 times more virus is produced in the cells exposed to the antiserum. The antiserum against hybrid cells containing only few human chromosomes is therefore active also against human diploid cells. Several independent immunisation experiments gave the same results.

The specificity of the anti-interferon effect was investigated after exhaustive adsorption of the antiserum on hybrid cells from lines either $4^+21^-22^+$ or $4^-21^+22^-$. Table 2 shows that the anti-interferon activity of the antiserum is lost only after adsorption on cells containing human chromosome 21. Furthermore, an antiserum prepared against mouse–human hybrid cells containing chromosome 6, 8 and X (but not 21) does not inhibit the antiviral effect of interferon even at high concentrations (experiment 2, Table 2).

Table 1 Effect of antiserum on action of interferon in hybrid cells

Additions	VSV yield in mouse–human hybrids WAVR4d (21$^+$) (log PFU ml^{-1})	JFA16a (21$^-$)
None	7.07	6.23
Interferon, 50 U	4.53	7.08
Anti-WAVR4d serum, 1:64	7.92	—
Antiserum + interferon	7.07	—

Interferon assays were carried out in microplates II (96 wells, Falcon) containing 2.5×10^4 cells per well in 0.1 ml Dulbecco's modified Eagle's medium—10% foetal calf serum (DVME). Incubations were at 37 °C. Antiserum adsorbed on mouse A9 cells (10^7 per ml serum) was added as indicated, followed after 1 h by human interferon. After 7 h, cells were washed and infected with 2.5×10^3 PFU VSV in Eagle's MEM–2% FCS. After 24 h, medium in each well was collected and the VSV titre was determined by the micromethod of Rosenthal and Shechmeister[14]. VSV titres are expressed in log PFU ml^{-1}. Karyotypes of the hybrid cells used were WAVR4d: 4,21,22; JFA 16a: 2,5,11,16,17,18 and 20.

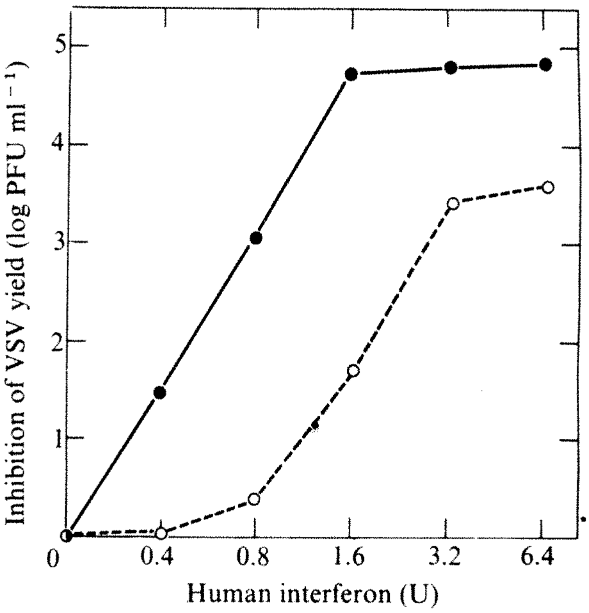


Fig. 1 Inhibition of the interferon effect by antihybrid serum applied to normal human diploid foreskin cells, FS7 (given by Dr J. Vilcek). Assay as in Table 1: to wells containing 2.5×10^4 cells in DVME–10% FCS, anti-WAVR4d serum (adsorbed on 10^7 mouse A9 cells per ml serum) was added at a dilution of 1/128. One hour later, the indicated amounts of interferon were added. After 7 h, cells were washed and VSV growth was measured as in Table 1. The virus yield in log PFU ml^{-1}, without interferon, was 7.28. The reduction in log PFU ml^{-1} VSV, caused by interferon, is plotted. ●, Control; ○, antiserum.

These data strongly implicate chromosome 21 as determining the antigen responsible for the serum's anti-interferon effect. We therefore compared the response of human fibroblasts either monosomic or trisomic for chromosome 21. The results

Table 2 Specificity of anti-interferon effect of antiserum for chromosome 21

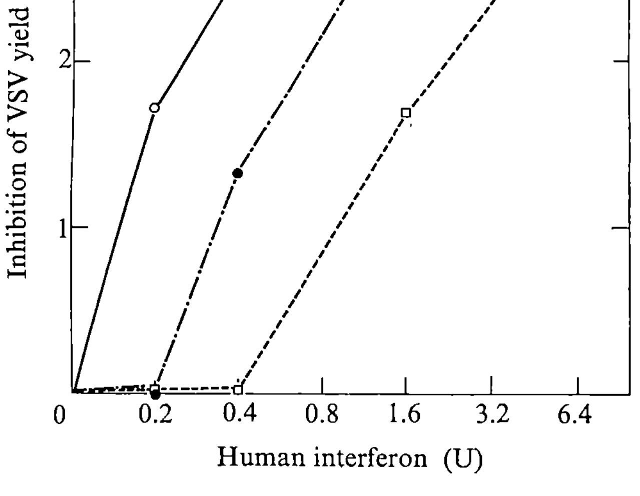
Antiserum used	VSV yield Inhibition by interferon (log PFU ml^{-1})	Antiserum effect
Experiment 1		
(1) No antiserum	3.6	—
(2) With anti-WAVR4d, 1:64 —adsorbed on mouse A9 cells	1.7	1.9
(3) —readsorbed on hybrid $4^+21^-22^+$ cells	1.7	1.9
(4) —readsorbed on hybrid $4^-21^+22^-$ cells	3.4	0.2
Experiment 2		
(5) No antiserum	1.9	—
(6) With anti-Hal-6 serum, 1:16	2.1	0.0
(7) With anti-WAVR4d serum, 1:16	0.0	1.9

Experiment 1: anti-WAVR4d serum was first adsorbed by incubation for 30 min at 25 °C with 10^7 mouse A9 cells per ml of serum diluted 1/32 in protein-free DVME. After centrifuging out the cells, antiserum was either used directly (line 2) or readsorbed at 2×10^7 cells per ml on mouse–human hybrid AIM3a (2,3,4,5,7,10,12,13,14, 17,19,22,X) (line 3) or AHA-16e (3,11,13,21,X) (line 4). Assay as in Fig. 1 with 1.6 U human interferon in 0.1 ml containing 2.5×10^4 human foreskin FS7 cells. Yield of VSV was 7.92 log PFU ml^{-1} without interferon. For experiment 2, mice were immunised with either mouse–human hybrids HAL (6,8,X) or WAVR4d (4,21,22) and the antisera obtained assayed as above. Yield of VSV was 7.08 log PFU ml^{-1} without interferon.

showed that the effect of the antiserum is stronger in monosomic 21 cells and decreases with increasing number of chromosomes 21 in the target cells. Because to produce the same inhibition in VSV replication, the monosomic line had to receive five times more interferon than the trisomic line[8] (Fig. 2), the antiserum effect is clearly determined by the cell and not by the interferon. Together with the fact that the hybrid cells WAVR4d, which served as immunogen, cannot produce human interferon (since they lack chromosomes 2 and 5) (ref. 9), these results enable us to conclude that the anti-interferon effect is due to antibodies directed against a cellular component different from interferon itself. This component, coded for by some gene(s) on chromosome 21, is required for the action of interferon.

Several observations indicate that the antiserum inhibits the action of interferon by combining with a component of the cell surface: (1) the effect is seen with living cells in which only the surface antigens are available for reaction with the antibodies[10]; (2) the inhibition is reversible: excess of interferon overcomes the inhibition caused by pretreatment of the cells with antibodies (Fig. 1); (3) immunofluorescence studies showed that the antiserum used in this work stains the surface of live human fibroblasts. We propose that this cell-surface component coded for by chromosome 21, serves as a receptor for human interferon. This interpretation is supported by the comparison of cells with different doses of chromosome 21. Figure 2 shows that chromosome 21 controls the amount of interferon needed to reach a given level of cell response, but not the final antiviral state achieved with saturating concentrations of interferon. This is expected if some gene on this chromosome codes for a receptor to human interferon; if, on the other hand, it would code for an intracellular antiviral protein (AVP) as suggested[8], the final antiviral state should be higher in trisomic than in monosomic 21 cells. In the mouse–human hybrids, the location of the gene for such an antiviral protein, made in response to interferon, remains undetermined; one possibility is that it needs not to be specifically human but may be a mouse gene.

Fig. 2 Comparison of human fibroblasts monosomic, disomic and trisomic for chromosome 21, in their response to human interferon. Lines GM 258 (trisomy 21) and GM 230 (monosomy 21) were obtained from the National Institute of General Medical Sciences genetic mutant cell repository (Camden). The diploid line was foreskin cells FS4. Interferon assay as in Fig. 1, except that treatment was for 24 h before VSV infection. The virus yield, after 29 h was in log PFU ml^{-1} 6.67 for FS4 and 5.38 for GM 258 and GM 230. ○, Trisomy 21; ●, diploid; □, monosomy 21



Human interferon binds rapidly to human cells[11]. Whether this first binding, which may involve gangliosides[12], triggers the antiviral state is still debatable[13]. We have found recently that even after 3 h of contact at 37 °C between interferon and human fibroblasts, the establishment of the antiviral state is still blocked by our antiserum. This indicates that a prolonged interaction of interferon with cell-surface receptors is necessary to induce the antiviral state. Our antiserum should prove useful for further study of cell–interferon interactions.

More generally, antisera against cell-surface determinants coded by given human chromosomes may select in a hybrid population against cells containing these determinants, and provide a selection method for the loss of specific human chromosomes.

We thank Mrs Claire Revel and Manette Dennis for assistance. M. R. was a recipient of an Eleanor Roosevelt fellowship from the International Union Against Cancer. We thank Dr R. Kucherlapati for helpful collaboration. This work was supported by grants from the National Institute of General Medical Sciences and the German Science Fund.

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Chromosome 21 and the cell growth inhibitory effect of human interferon preparations

INTERFERONS constitute a class of biologically active glycoproteins[1–3] which induce a spectrum of physiological changes in living cells, leading to the inhibition of virus replication[4], of cell growth *in vitro*—both normal and tumour cells[5,8]—and of tumour growth *in vivo*[9–15]. The mechanism(s) underlying these effects is unknown. Gressor *et al.* have suggested that the same molecule in an interferon preparation is responsible for both the antiviral (AV) and cell growth inhibitory (CGI) effect[5]. This suggestion was based on the observation of a consistent and direct correlation between the two effects in an interferon preparation purified more than a millionfold. Gressor also reported that tumour cells treated with interferon had a lower capacity to form colonies in Agarose and were less tumorigenic when innoculated[15], suggesting that the antitumour effect of interferon depended, in part, on the inhibition of tumour cell multiplication which can be demonstrated *in vivo*. I describe here a genetic approach to ascertain whether the CGI effect of human interferon preparations is mediated by chromosome 21-directed gene(s), known to govern the AV action of interferon[16–19]. Such an approach, should provide further insight into whether the different effects of interferon are governed by a cluster of genes on chromosome 21 or whether a single genetic locus on chromosome 21 codes for the pleiotropic effects of interferon.

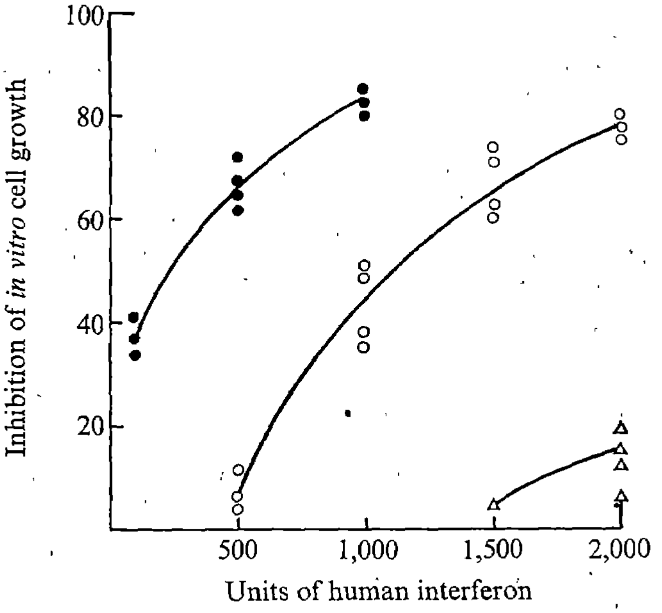

Fig. 1 Effect of human leukocyte interferon on the *in vitro* cell growth of trisomic 21 (●), disomic 21 (○) and monosomic 21 (△) human skin fibroblasts. Each point on the graph represents the results from one determination.

The CGI and AV effects of two human interferon (HIF) preparations were assayed on primary human skin fibroblasts containing different copies of chromosome 21. These cells are monosomic (M21), disomic (D21) and trisomic (T21) for chromosome 21. Primary human cells carrying chromosomal translocations were also used: (1) a diploid cell line (t4;21), in which one of the two chromosomes 21 is translocated to the short arm of chromosome 4, with the resultant loss of the proximal short arm of chromosome 21 (ref. 20); (2) a trisomic 21 cell line (t6;21), in which one of its three chromosomes 21 is translocated to chromosome 6, with the resultant loss of the centromere as well as the short arm of chromosome 21 (ref. 21), (3) another diploid cell line (t4;2), carrying a 2,4 chromosomal translocation, and (4) a trisomic 21 cell line (T21;t–17), carrying a 1–17 chromosomal translocation[22]. A primary human skin fibroblast trisomic for chromosome 18 is also used. The CGI and AV effects of interferon were assessed in these cells by assaying the amount of interferon required to inhibit the *in vitro* growth of cells and synthesis of vesicular somatitis virus, respectively, by 50%. Human cells which were less sensitive to the CGI effects of interferon were also less sensitive to its AV action (Table 1), confirming a similar observation in L1210 cells with differential sensitivity to mouse interferon[23]. Table 1 also shows that the sensitivity of human cells to both the CGI and AV actions of interferon varied inversely with the number of copies of human chromosome 21 present in the cell. Similarly, Fig. 1 shows that M21 cells were less sensitive than D21 cells, which in turn were less sensitive than T21 cells to the CGI effect of interferon. It is unlikely that these observations were due to a nonspecific increase in the number of human chromosomes because the T18 cells, which contain an extra copy of chromosome 18 but have the normal complement of chromosomes 21, behave like a D21 cell in terms of sensitivity to the CGI effect. These results suggest that chromosome 21 contains the gene(s) governing both the CGI effect and the AV action of interferon. Table 1 also shows a dose effect of translocated chromosome 21 on CGI activity. This translocated dose effect has been used to assign the gene(s) governing the AV action of interferon to the long arm of chromosome 21 (ref. 24). The translocated 21 chromosomes (the long arm portions) in both the t4;21 and t6;21 lines exert a dose effect on these cells such that t4;21 and t6;21 cells were as

Table 1 Response of cells to interferon

Cell type	Identification no.	Units of HIF inhibiting by 50%			
		Cell growth		Viral RNA synthesis	
		NIH-1F	108-1F	NIH-1F	108-1F
M21	230	>2,500	>2,500	1.8	1.8
	198	>2,500	>2,500	1.7	1.6
D21	141	1,664, 1,860	800, 1,100	0.42	0.48
	115	1,760, 1,902	825, 985	0.48	0.50
	15	1,458, 1,264	924, 1,024	ND	ND
T21	258	256, 240	40, 80, 95	0.11	0.10
	114	212, 236	64, 112, 118	0.12	0.14
	14	224, 301	112, 116, 142	ND	0.09
T18	143	1,560	860, 1,114	0.50	ND
t4; 2	301	1,811, 1,763	1,030, 1,004	0.44	0.45
t4; 21	98	1,988, 2,010	1,306, 1,114	0.41	0.40
t6; 21	144	255, 276	48, 36, 72	0.08	0.09
t21;1–17	201	199, 288	42, 134, 104	0.09	0.12

NIH-1F, Sendai-induced human leukocyte interferon reference reagent G023-901-S-27.

108-1F, poly(I)·poly(C)-induced human fibroblastoid interferon from a high producing cell line V-M15-clone 108.

For the cell growth inhibitory assay, 2×10^4 cells were seeded per 25 cm^2 flask and maintained in 5 ml of regular growth medium (MEM medium containing 10% of heat-inactivated foetal bovine serum, 2 mM glutamine and 1% chlorotetracycline) for 20–24 h. The test flasks were reincubated with different concentrations of human interferon in 5 ml of regular medium. The cultures were incubated at 37 °C for 6 d. On day 6 cultures were trypsinised and total cells were counted with a Coulter counter The CGI effect was assessed by the amount of HIF required to inhibit the total cell count by 50%. Control cultures were treated the same as the test cultures, except that interferon was omitted. Each figure represents average results obtained from one set of duplicate cultures.

sensitive as D21 and T21 cells, respectively, to the CGI activity of interferon (Table 1). It is unlikely that the effects observed with these translocations were due to a nonspecific chromosomal translocation, because two other translocations—the 4–2 translocation in the t4,2 line containing two copies of chromosome 21 and the 1–17 translocation in the T21 cells (T21,1–17) line containing three copies of chromosome 21—behaved like D21 and T21 cells, respectively, in terms of their sensitivity to the CGI effect. The results suggest that the CGI effect like the AV action of interferon is governed by gene(s) located on the long arm of chromosome 21.

Table 1 shows that for each unit of AV activity, human fibroblastoid interferon (108-1F) was more inhibitory to growth than human leukocyte interferon (NIH-1F) This is not surprising since the two interferons are biologically and antigenically different[25–27]. But whether this means that human fibroblastoid interferon is potentially more effective than leukocyte interferon as an antitumour agent is subject to further study.

Of the two interferons used in this study, 108-1F was a poly(I)·poly(C)-induced interferon from our high producing line of human aneuploid cells containing multiple copies of the chromosomes associated with interferon production[19,28]. To eliminate the possibility that the CGI effect observed with this preparation was due in part to residual poly(I)·poly(C), cells were grown in medium containing the concentrations of poly(I)·poly(C) indicated in Table 2. There was no inhibition of cell growth at these concentrations of poly(I)·poly(C), rather, at the lower concentrations, a small stimulation of cell growth was apparent.

Table 2 Effect of poly(I)·poly(C) on growth of primary human skin fibroblast cultures

poly(I)·poly(C) (μg ml^{-1})	Total cell count of cultures Treated	Not treated
0.09	1.1×10^5	1.3×10^6
0.05	1.3×10^5	$0\,9\times10^5$
0.009	1.4×10^6	1.1×10^5
0.001	1.2×10^5	$0\,8\times10^5$

Human cells trisomic for chromosome 21 (line 114) were seeded at 2×10^4 cells per 25 cm^2 flask and maintained in regular growth medium for 20–24 h. The medium was removed from each flask and reincubated with growth medium containing the above concentrations of poly(I)·poly(C) (P.L. Biochemicals) for 6 d. Poly(I)·poly(C) was omitted from controls. Each figure represents average counts from one set of duplicate cultures.

To interpret the significance of these findings, a model is presented to relate the role of chromosome 21 to the pleiotropic effect of interferon. According to this model, a single gene or a cluster of genes located on the long arm of chromosome 21, govern(s) the response of human cells to interferon. One of the first events after the treatment of cells with interferon is the interaction between interferon, or an inhibitory growth substance which has been coinduced and/or coreleased with interferon, with their respective receptor sites. These sites could also be specified by the chromosome 21-directed genes. (There is no direct evidence for the existence of these receptor sites, although they have been inferred from studies with interferon bound on Sepharose beads[29].) Then the chromosome 21 gene(s) is stimulated, presumably by the events at the cell surface[18,18,30], to transcribe gene product(s) which in turn are directly responsible for mediating the pleiotropic effects of interferon. Evidently, a newly induced second protein is implied here But unlike the induction of the AV state, there is no evidence that a second protein is required for the CGI effect. For this reason an alternative explanation is offered Presumably, again, interferon or the inhibitory growth factor interacts with a chromosome 21-directed gene product(s). This interaction produces chemical and/or conformational change(s) which interfere(s), with the replication of viruses and the normal rates of cell division. For this alternative, it is necessary to postulate that these gene product(s) are synthesised constantly and turned over rapidly. This assumption then explains the inhibition of the AV effect by actinomycin D (ref. 31) as well as the lack of the AV induction in enucleated cells[32]. Another alternative is that an interaction between interferon or inhibitory growth factor and chromosome 21 gene(s)-directed cell-surface component(s) is sufficient to cause cell-surface changes. These in turn inhibit the normal rates of cell division but not viral replication, which requires a second protein, the AV protein. To decide between these alternatives, it is essential to identify and isolate the gene product(s) in question. Until then, it would be worthwhile to consider these alternatives equally.

A natural sequel to this is to apply a similar approach to ascertain whether chromosome 21 also controls the antitumour effect of human interferon. As might be anticipated from the suggestion that the antitumour effect depends in part on the CGI effect, it will be feasible to design *in vitro* tests using this effect to predict the human tumour types that will be most responsive to interferon therapy.

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Received November 17, accepted December 12, 1975.

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Spermatogenic failure of translocation heterozygotes affected by *H-2*-linked gene in mouse

ACCORDING to Lifschytz and Lindsley[1,2], the basic control mechanism of spermatogenesis of all male heterogametic organisms consists of inactivation of the X chromosome during differentiation of germ cells The absence of this control mechanism in female meiosis, where both X chromosomes are active[3,4], could explain why only male gametogenesis is impaired in the carriers of some chromosomal aberrations.

Here, the first data are reported on a genetic factor located on the 17th murine chromosome which controls the range of spermatogenic impairment of mice heterozygous for T(14,15)6Ca translocation[10] (abbreviated, T6).

I have previously envisaged[5] a possible mechanism which would relate the male sterility of autosomal translocation carriers to the impairment of X-chromosome inactivation. The hypothesis of Lifschytz and Lindsley[1,2] fits well with the data on spermatogenic breakdown caused by X-autosomal translocations and certain X-chromosome deficiencies[1,2] but it apparently could not account for sterility caused by many autosomal reciprocal translocations[6-8]. Nevertheless, several characteristic features of both types of translocation indicate a possible common mechanism of male infertility: (1) the sterility is clearly chromosomal in both types of translocation heterozygotes; (2) only male carriers are sterile; and (3) infertility is caused by the breakdown of spermatogenesis.

My idea[5], outlined above and based on the observation of a non-random tight association of the X chromosome with translocation multivalent in primary spermatocytes, has been supported by an analogous cytological picture found in one tertiary trisomic male mouse[9] and in the other male-sterile translocation (J.F., unpublished). These data, however, give little information on the causal relationship between the X-autosomal attachment in meiosis and the subsequent spermatogenic failure Thus, another approach to the analysis of sterility of autosomal translocation carriers, might seem desirable.

The genic component of variation in the severity of spermatogenic failure of males heterozygous for T6 translocation is revealed when the particular types of F_1 hybrids from crosses of CBA–T6/T6 males with females of nine inbred strains are compared (Table 1). The symbol CBA–T6/T6 designates a homozygous T6 translocation carried on the genetic background of CBA inbred strain. By comparing the distributions of testes weight values, which are sensitive measures of spermatogenic impairment[11-13], the three phenotype classes of testes weight values (high, medium and low) are distinguished among the nine types of F_1 hybrids. The mean number of spermatozoa in epididymides, which is another criterion of spermatogenic failure[14], shows reasonable correlation with testes weight in spite of variations in individual scores (Fig. 1)

Table 1 shows further that spermatogenic failure displays several attributes of hybrid sterility[15]; the two homozygous parental forms, CBA/J and CBA–T6/T6, are normally fertile, the impairment of gametogenesis being limited only to T6 heterozygous male progeny of a CBA/J×CBA–T6/T6 cross. This combination is particularly interesting as the two parental strains, CBA/J and CBA–T6/T6 are genetically (quasi-)identical, except for a single reciprocal translocation between chromosomes 14 and 15. Thus, spermatogenic failure of the F_1 hybrids can be entirely ascribed to the heterozygosity for this chromosomal rearrangement.

Table 1 Testes weights and sperm counts in T6 heterozygous male progeny of (inbred strain×CBA–T6/T6) cross

Inbred strain*	Mean testes weight† (mg)	s.e.	Sample size‡ T/S	Number of spermatozoa§ $\times 10^{-4}$
C3H/Di	154 (H)	2.3	13/7	158
A/Ph	120 (M)	2.5	14/14	120
BALB/c	119 (M)	1.8	13/6	133
F/St	119 (M)	2 2	8/0	ND
I/St	125 (M)	2.1	20/9	178
AKR/J	99 (L)	3.9	12/12	97
C57BL/10	98 (L)	1.1	54/27	49
C57BL/6	98 (L)	2.7	19/7	16
CBA/J	93 (L)	3.6	11/8	40
Homozygous (T6/T6 and +/+) control males				
CBA–T6/T6	138	2 0	16/14	221
CBA/J	143	4.5	16/16	245

*Female parent.

†Wet weight of paired testes of males aged 60±4 d; H, M, L, high, medium and low testes weight.

‡Number of F_1 hybrid males examined for testes weight (T) and those examined for sperm counts (S).

§Mean number of sperm heads in paired epididymides.

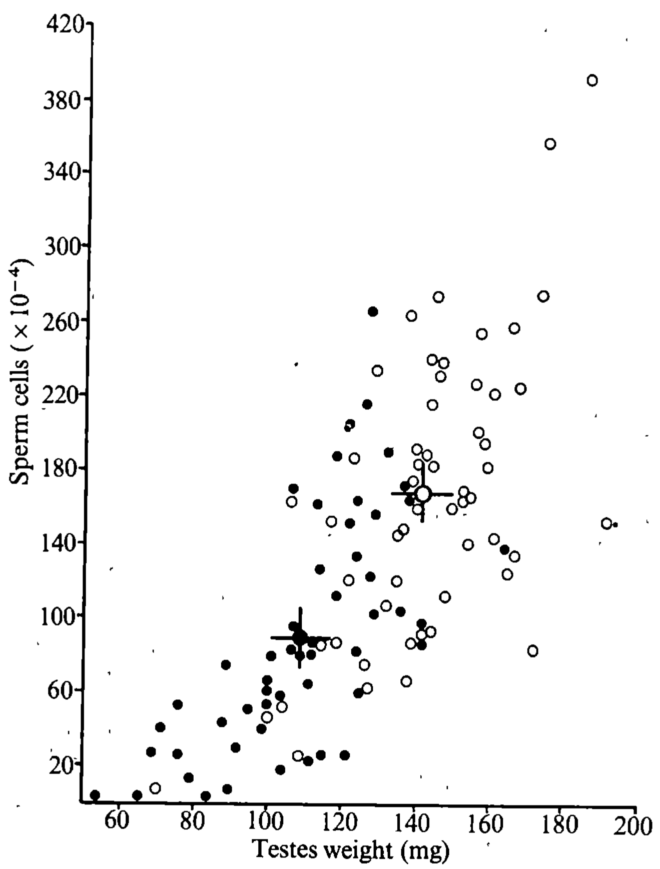

Fig. 1 Number of spermatozoa in paired epididymides plotted against testes weight in T6 heterozygous male progeny of CBA–T6/T6×(C57BL/10-*T*×C3H)*T*/+ cross. The tested males, 60±4-d-old, were genotyped as $H\text{–}2^b/H\text{–}2^k$ (●) or $H\text{–}2^k/H\text{–}2^k$ (○) by PVP haemagglutination[21] with specific anti-$H\text{–}2^b$ sera. The mean values of $H\text{–}2^b/H\text{–}2^k$ males (crossed ●), (109 mg, 90×10^4 sperm cells) and $H\text{–}2^k/H\text{–}2^k$ males (crossed ○), (141 mg, 164×10^4 sperm cells) differ significantly (Hotellings T^2 statistics=31.762; $P<0.01$) which indicates that a gene linked to $H\text{–}2^b$ on chromosome 17 from C57BL/10-*T* strain is the major genetic determinant responsible for low testes weights and low sperm counts. Fourteen recombinants between *T* and *H–2* were detected among 107 males tested; however, they do not provide sufficient information for location of the postulated gene with respect to *T* and *H–2* loci, partly because of a physiological component in variation of fertility parameters, and partly because of difficulties with classification of some *T*/+ compared with +/+ animals on this particular genetic background. Therefore, for the sake of clarity, only the *H–2* genotype is shown.

Chromosome 17 of the mouse carries at least two gene loci involved in a similar type of male-limited sterility, that is, the sterility factor of *t* alleles[16,17] and the *Hst-l* gene[13]. Using the *H-2* complex and *T* allele (*Brachyury*) as markers of chromosome 17, a distinct part of genetic variation in spermatogenic failure of T6 heterozygotes can be ascribed to a gene (or a gene cluster) linked to these markers on chromosome 17. The conclusion is drawn from an analysis of male progeny of CBA–T6/T6×(C57BL/10-*T* ×C3H)*T*/+ cross. Those males that received, from their F_1 parent, the $H\text{–}2^b$ haplotype and/or the *T* allele (the two markers of chromosome 17 of C57BL/10-*T* strain) had significantly lower testes weights and sperm counts than their brothers with H-2^k and $+^T$ markers of C3H origin (Fig. 1).

The relationship of the described gene to *T*/*t* and *H-2* complexes and to the *Hst-1* gene is not quite clear; it seems unlikely that the *T* allele itself would be involved since *T*/+ and +/+ male progeny of the CBA-T6/T6× C57BL/10-*T* cross does not differ in testes weights Furthermore, the strain distribution shown in Table 1 does not fit the assortment of alleles either of the *Hst-1* gene[13] or of the *H-2* complex; in this case, however, the possibility cannot be excluded that the genetic background in some strains can alter the expression of the described gene

Although the precise location of the described gene on chromosome 17 might have a bearing on the concept of the *T*/*t*–*H-2* supergene[18–20], two other questions that emerged from this preliminary genetic analysis are those concerning the effect of the described gene on further male-sterile translocations, and the role of this gene in the presumed mechanism[5] of translocation-associated male sterility.

I thank Dr P. Iványi for discussions, and Mrs J. Susová and Miss E. Kozlová for assistance.

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Received October 7, 1975; accepted January 21, 1976.

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Facilitation or attenuation of mixed leukocyte culture responsiveness by adherent cells

It is generally agreed that adherent cells, or macrophages, are required for maximum reactivity in mixed leukocyte culture (MLC). The system has been incompletely defined, for the mode of function of the adherent cell is unclear. There is evidence from human and animal systems that adherent cells have either a nonspecific helper role[1–4] or a more precise genetically defined stimulator role[5]. We report here that adherent cells which ingest latex particles seem to be capable of nonspecifically facilitating or attenuating human lymphocyte proliferation in MLC.

Heparinised venous blood was subjected to Ficoll–Hypaque flotation to obtain mononuclear cells. An aliquot of these cells was filtered through columns of Sephadex G-10 beads according to the method of Ly and Mishell[6], the principal modification being the use of medium containing 20% foetal calf serum for elution. This procedure selectively depletes the mononuclear cells of adherent latex-ingesting cells while leaving the relative proportion of T and B lymphocytes unchanged (Table 1). In these experiments, we accounted for 88–103% of the mononuclear cells using conventional cell marker techniques. The mean yield of lymphocytes from this column was 93%, and the viability was greater than 99% by Trypan blue exclusion. Thus, the only discernible effect of this procedure was selectively to remove adherent cells which ingest latex particles.

Using well defined methods[7], one-way MLCs involving 5×10^4 responding cells and 5×10^4 X-irradiated stimulating cells were planted in microtrays in 150 μl of RPMI-1640 containing 20% pooled normal human serum, HEPES buffer (25 mM), penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}), and fresh glutamine (2 mM). In all experiments, only fresh cells were used. MLCs were cultured for 132 h in a humidified atmosphere of 5% CO_2 and were labelled for the last 16 h of culture with 2.0 μCi of ^{3}H-thymidine. Cultures were collected on to glass-fibre filters with a semi-automated sample harvester, and ^{3}H-thymidine incorporation was determined by liquid scintillation spectroscopy The incorporation of isotope was expressed as the mean c.p.m of triplicate allogeneic cultures minus the mean c.p.m. of the isogeneic control.

Figure 1 clearly demonstrates that removal of adherent latex-ingesting cells either isogeneic to the stimulator or isogeneic to the responder has no significant effect on MLC reactivity ($P > 0.20$ by two-tailed *t* test). Removal of adherent cells from both the stimulating and responding cell populations, however, results in significantly decreased MLC reactivity ($P > 0.05$ by one-tailed *t* test), in spite of the fact that the relative proportions of T and B lymphoctyes in the column-filtered cells were virtually unchanged. It must be noted that these MLCs involved allogeneic combinations of HLA dissimilar individuals who were mutually strongly responsive in the routine MLC.

Abnormally low responsiveness in MLC can be quite

Fig. 1 *a–c*, The response of filtered cells of 14 normal individuals in MLC Each point represents the mean of 3 separate MLCs and is expressed as the percentage of the mean of the 3 companion unfiltered MLCs. Response of a single individual's filtered cells to 3 separate allogeneic stimuli is 81–124% of the unfiltered response (*a*). The mean response of 3 separate individuals' cells to a single filtered allogeneic stimulus is 88–121% of the unfiltered response (*b*). The mean response of a single filtered cell population to 3 separate filtered allogeneic stimuli is attenuated (*c*). The increased responsiveness of patients' filtered lymphocytes to 4 separate allogeneic stimuli relative to the unfiltered responses (*d*).

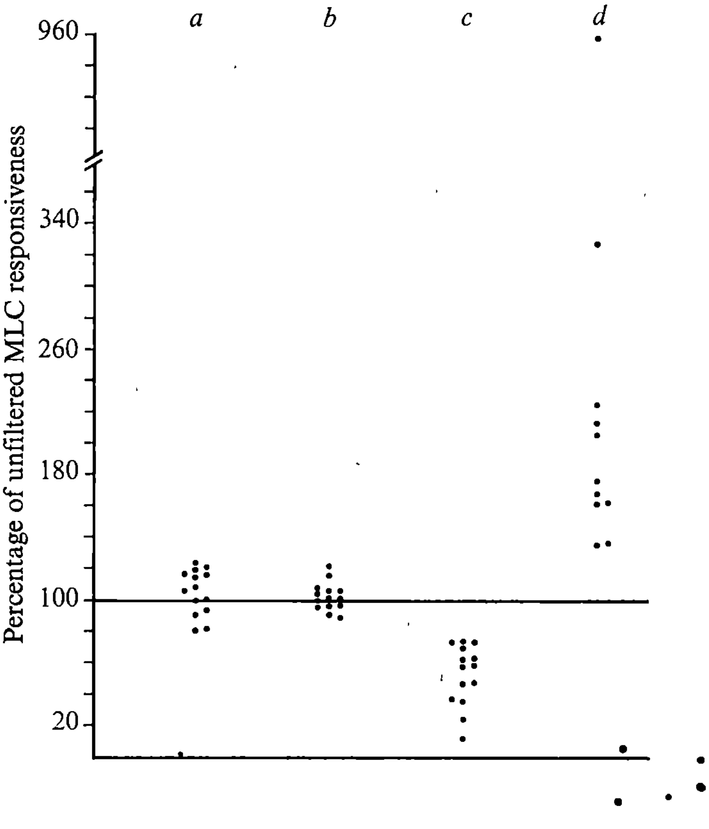

Table 1 Cell marker analysis of filtered and unfiltered cells

Subject	Lymphocytes identifiable as T cells (%)		Lymphocytes identifiable as B cells (%)		Mononuclear cells identifiable as macrophages (%)	
	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	Filtered
1	68	75			8.0	0
2	70	74			4.5	0
3	80	74			5.5	0
4	66	73			5.5	0.25
5			6	4	6.0	0
6			16	18	5.5	0
7			15	11	16.5	0
8			14	12	11.0	0
9			10	9	13.0	0
10	72	78	20	14	6.5	0.5
11	83	82	18	13	3.7	0
12	88	88	9	6.6	11.5	0
13	77	75	15	15.5	9.0	0
14	85	80	11	8	7.0	0
15	87	80	12	10	8.5	0.7

All percentages represent a minimum of 300 cells counted. T lymphocytes were determined by their ability to rosette spontaneously with sheep red blood cells. B lymphocytes were enumerated with a rhodamine-conjugated polyvalent anti-human immunoglobulin antiserum. Macrophages were identified by their ability to phagocytose latex particles 0.801 μm in diameter.

reliably identified by calculating a relative response (RR)[8] for an individual being tested: This value expresses the responsiveness of an individual's cells to a panel of four separate allogeneic stimuli, relative to the responses of several normal individuals to the same allogeneic panel. We have determined an RR for each of 33 normal individuals. The range was 79–136% with a geometric mean RR of 101%. An abnormally low RR was considered to be one greater than two standard deviations (2σ) below this mean (μ), namely, less than 76%. These calculations involved logarithmic transformations of the data since expression of these values as relative changes, by definition, generated a log normal distribution.

Eleven individuals demonstrating abnormally low responses in MLC were selected from a group of patients with non-lymphoid malignancies. Six of these individuals demonstrated RRs $>3\sigma$ ($<66\%$) below μ, and the other five demonstrated RRs $\geqslant 5\sigma$ ($<50\%$) below μ. Figure 1 shows that filtered lymphocytes from these individuals, which were virtually devoid of adherent latex-ingesting cells, demonstrated significantly increased proliferative capacities in response to each of four normal allogeneic stimuli compared with the companion MLCs using unfiltered patient responding cells. When column-filtered lymphocytes were used, the RRs of eight of these individuals returned to values within the normal range as defined above, while the remaining three returned to near normal (data not shown). This markedly increased responsiveness was significantly different from that observed with 56 normal allogeneic combinations involving filtered responding cells ($P<0.005$ by one-tailed t test). In addition, four of these patients demonstrated a significantly decreased ability to stimulate normal allogeneic cells. When the patients' adherent cells were removed from the stimulating population by column filtration, the proliferative capacity of the four normal responders returned to normal values.

Our findings with mononuclear cells of HLA dissimilar normal individuals confirm the findings of others[2,9,10] that adherent cells are necessary for optimal MLC responsiveness whether the adherent cell is isogeneic or allogeneic to the responding cell population. Depletion of these cells from both the stimulating and responding cell populations results in significantly decreased MLC responsiveness. In addition, in the pathological situation, the adherent cell can be responsible for attenuated lymphocyte blastogenic activity, and when removed from culture, normal or near normal lymphocyte proliferation may be observed. Our experiments strongly suggest that such increased proliferation of patient lymphocytes is mediated by the allogeneic adherent cells in the normal irradiated stimulating cell population, giving further testimony to the nonspecific effects which mononuclear phagocytes may display in MLC.

In the light of the findings that macrophages can secrete discrete factors which are either stimulatory or inhibitory for cellular proliferation[11], it is appropriate to speculate that in human malignancy a perturbation of macrophage function occurs which involves a net increase of an inhibitory influence during the response to an allogeneic stimulus.

This work was supported by a grant from National Institutes of Health.

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Received November 21, 1975, accepted January 16, 1976.

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Isolation of a subpopulation of adherent peritoneal cells with anti-tumour activity

We have reported that adherent peritoneal cells from normal mice stimulated the growth of many lymphomas *in vitro*, while adherent peritoneal cells from mice injected with Bacille Calmette-Guerin (BCG) were far less stimulatory[1]. The latter were overtly cytostatic when tested on L929 cells[1], as well as on several other adherent fibroblastic malignant targets (unpublished). This leads us to speculate that adherent murine peritoneal cells consist of at least two subpopulations, one that tends to stimulate and one that tends to inhibit the proliferation of susceptible target cells. According to this view, infection of the mouse with BCG might lead to increased numbers or activity of the cytostatic subpopulation at the expense of the stimulatory subpopulation. We have now isolated an adherent, esterase-positive, poorly phagocytic mononuclear peritoneal cell with potent tumour-inhibitory capacity, which may represent the postulated cytostatic subpopulation.

Cells were washed from the unstimulated peritoneal cavities of DBA/2 or CDF1 mice or from age-matched mice 1–21 weeks after intraperitoneal injection of 10^7 viable BCG of the Phipps or Pasteur strains (Trudeau Nos 1029 or 1011). The cells were washed and allowed to adhere to plastic 35-mm dishes (Falcon) for 2–3 h in RPMI-1640 with 20% foetal calf serum. The dishes were then rinsed vigorously in beakers of saline, leaving cells which were essentially all esterase positive[1]. Starch granules prepared from the seeds of *Amaranthus caudatus*[2] were added to the monolayers in conditions leading to maximal phagocytic uptake, as defined for these cell preparations in preliminary experiments with ^{14}C-acetyl-labelled granules by methods described previously[3,4]. At the conclusion of the phagocytic period, the dishes were again washed vigorously in six beakers of saline.

In conditions of maximal uptake (15–20 mg of starch per dish for 3 h), microscopy revealed that $4.8\pm0.5\%$ of the adherent peritoneal cells in the monolayers from normal mice and $16.8\pm1.3\%$ of those from BCG mice were non-phagocytic for starch (mean ± s.e. for 14 and 17 experiments, respectively; "non-phagocytic" defined as 0–1 cell-associated granule). Cells were then dislodged from the monolayers by incubation in medium containing lidocaine[5] (Astra Pharmaceuticals) followed by exposure to jets of saline from a syringe. This procedure recovered nearly all cells from the normal monolayers but left behind a variable number of non-phagocytic cells in the dishes prepared from BCG-treated mice. The cells were washed, spun on Ficoll–Hypaque[6] at 240*g* for 12 min, and the non-sedimenting cells were washed again. Phagocytic cells and free starch pelleted, leaving at the interphase a preparation of non-phagocytic mononuclear cells with a viability of $92.4\pm1.9\%$ by Trypan blue exclusion, contaminated with $3.5\pm1.3\%$ of viable cells containing more than one starch granule, and with $0.009\pm0.0004\%$ of the initial free starch granules (mean ± s.e. from 14 to 17 preparations).

These non-phagocytic cells were plated in microtest wells (Falcon) in 0.2 ml with various tumour cells for 2 d, as indicated in Table 1. Uptake of ^{3}H-thymidine during the last 4 h of culture was measured as described before[1]. In each of five experiments, the isolated non-phagocytic adherent cells inhibited the uptake of ^{3}H-thymidine by the tumour targets to a greater degree than similar numbers of the total adherent peritoneal cell populations from which they were derived. The non-phagocytic adherent cells from BCG-treated mice appeared to differ from those from normal mice only in that there were nearly four times as many of them, and they were apparently more resistant to dislodgement by lidocaine. In dose–response experiments (not shown), there was no evidence that, per recovered cell, they were more potent. As few as 2.5×10^3–5×10^3 cells of the non-phagocytic subpopulation from either the BCG or the normal adherent populations markedly inhibited the uptake of ^{3}H-thymidine by 1×10^3 MCA-3 cells. Controls showed that the addition of starch and lidocaine to peritoneal cells in suspension with rapid centrifugation over Ficoll–Hypaque, before phagocytosis had proceeded very far, gave preparations which behaved like the parent populations (Experiment 2, Table 1). Likewise, when peritoneal cells were

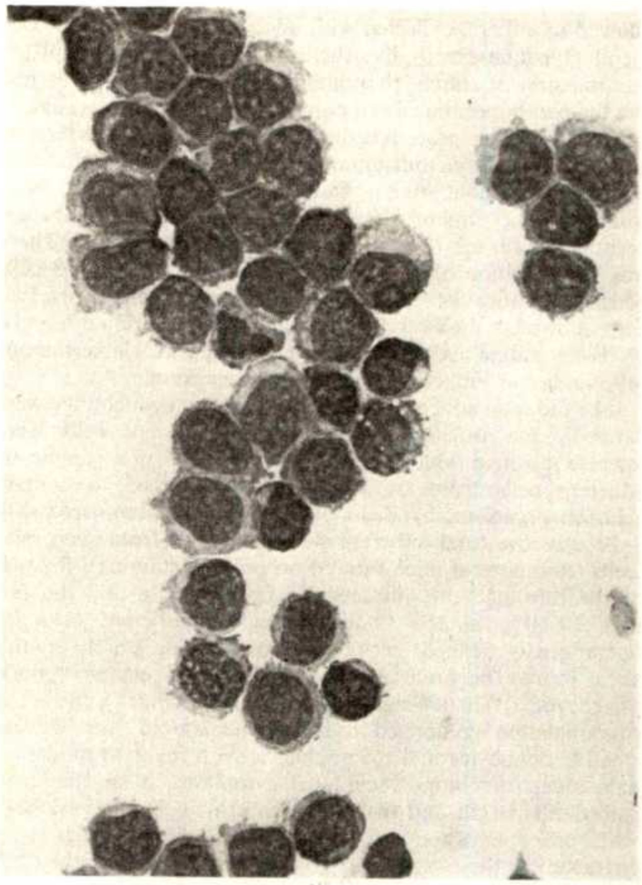

Fig. 1 Cytocentrifuge preparation of non-phagocytic adherent peritoneal cells prepared from untreated mice, as described in the text. (Wright's stain; magnification ×880.)

Table 1 ^{3}H-thymidine uptake after 2-d culture of tumour cells with adherent peritoneal cells (c.p.m., mean ± s.e.†)

Experiment	Tumour*	No. of tumour cells plated	Tumour alone	With total adherent cells‡ Normal	With total adherent cells‡ BCG	With subpopulation‡¶ Normal	With subpopulation‡¶ BCG
1	MCA-2	1×10^3	2,310±87	10,229±1,017	1,332±103	ND	0±141
		5×10^3	1,280±104	5,847±559	651±157	ND	0±50
2	MCA-3	1×10^3	4,996±158	5,766±492 (7,476±527)§	2,976±376 (3,266±151)§	319±13	267±54
3	MCA-3	1×10^3	1,999±84	4,319±463	1,480±37	29±8	99±15
	L929	1×10^3	4,020±153	7,393±462	2,744±66	74±15	25±6
4	MCA-3	1×10^3	1,567±25	3,121±334	1,134±49	181±24	329±31
		5×10^3	10,070±896	15,352±897	4,495±516	1,243±71	ND
5	MCA-3	1×10^3	2,507±85	2,935±310	1,418±39	277±65	381±46
		5×10^3	7,525±48	13,200±533	3,679±254	804±103	778±43

* MCA-2 and MCA-3 are tissue culture lines derived from methylcholanthrene-induced sarcomas of the C57BL/6 mouse. L929 cells were obtained from Microbiological Associates.

† c.p.m. are corrected for the c.p.m. taken up by each group in the absence of tumour cells. Mean background values for each column from left to right above were: 33±5 (no cells: machine background); 291±109; 120±66; 45±10; 42±18.

‡ For total adherent cells, 5×10^4 peritoneal cells were added to each well for 3 h and non-adherent cells were removed before addition of tumour cells, leaving an average of 2×10^4 adherent peritoneal cells[1]. For the subpopulation, 2×10^4 cells were used in each well. Values are for triplicate cultures.

§ Peritoneal cells treated with lidocaine and starch in suspension before being processed over Ficoll–Hypaque.

¶ After phagocytosis, monolayers were treated with 36 mM lidocaine for 2 min (Experiments 1 and 2) or 12 mM lidocaine for 5 min (Experiments 3 and 5). The latter gave better yields.

allowed to adhere, collected with lidocaine and processed over Ficoll–Hypaque exactly like the experimental cells, except for the omission of starch, they interacted with tumour cells just like the parent peritoneal cell populations. These results suggest that the isolation procedure itself did not induce anti-tumour activity. In addition, anti-tumour activity could not be induced by addition of contaminating amounts of free starch to wells containing the tumour alone or tumour with either adherent peritoneal cells or the non-phagocytic subpopulation. There was no inhibition of uptake of ^{3}H-thymidine in MCA-3 cells added to cultures of non-phagocytic adherent cells which had been allowed to die by 4–5 d of preculture. Inhibition of uptake of ^{3}H-thymidine accurately reflected changes in viable tumour cell number as judged by haemocytometer counts.

Like the total adherent cell population from which they were derived[1], the isolated non-phagocytic adherent cells were esterase positive. Between 0.3 and 10% of non-phagocytic adherent cells from BCG-treated mice seemed to contain acid-fast organisms by Ziehl–Neelsen staining, compared with 3–10% of the total adherent peritoneal cells from such mice (cells from normal mice showed no positive staining). Because of the rare stainable intracellular mycobacteria and the fact that 10–50% of the "non-phagocytic" adherent cells (as operationally defined) seemed to contain one starch granule each, it may be preferable to regard these cells as "poorly phagocytic". When Wright's stain was used (Fig. 1), the active subpopulation comprised cells intermediate in size between small lymphocytes and macrophages, with scant to moderate, pale blue, sometimes vacuolated cytoplasm, a spiculated or ruffled cell margin, and an oval to reniform nucleus, occasionally with prominent nucleoli. Further work is in progress to characterise these cells; it is not clear whether they are macrophages.

Allison described non-phagocytic, adherent murine peritoneal exudate cells which he suggested might be effector cells in antibody-dependent cell-mediated cytotoxicity[7]. Cells with some similar characteristics were found in mouse spleen by Greenberg *et al.*[8]. Boyle and Ormerod isolated a subpopulation of phagocytic, adherent peritoneal cells from alloimmunised mice which seemed to bear all the cytotoxic potential for the specific tumour cell target[9]. Subpopulations of macrophages with different activities in endocytosis[10,11], immune RNA production[11], or antigen binding[12] have been obtained using density centrifugation in Ficoll[10,12] or albumin[11]. It is not yet known whether there is any relationship between the subpopulations described by these workers and the subpopulation described here.

Because of the metabolic perturbations induced by phagocytosis itself[13,14], the separation procedure used here precludes testing the residual, phagocytic cells in their basal state. Other techniques will be needed before the non-phagocytic adherent subpopulation can be compared directly with the remainder of adherent peritoneal cells in an anti-tumour assay.

Work is in progress to test the hypothesis that different classes of tumour cells differ in their susceptibility to the postulated inhibitory and stimulatory subpopulations of adherent peritoneal cells.

Thus, among adherent murine peritoneal cells, there are esterase-positive cells which can be isolated in nearly pure form on the basis of their deficient phagocytosis. These cells markedly inhibit the replication of various tumour cells *in vitro*, and seem to account quantitatively for the inhibition afforded by the total adherent peritoneal cell population from which they were derived. The tumour-inhibitory subpopulation can be isolated from untreated mice, although it can be identified in greater number among the adherent peritoneal cells from mice given BCG. The anti-tumour action of the subpopulation seems to be nonspecific. The existence of such a subpopulation may necessitate reassessment of the relationship of the numerous features of "macrophage activation", measured in a whole population, to its anti-tumour activity.

We thank Dr R. Hodes for helpful discussions, Drs S. A. Rosenberg and G. Parker for MCA-2 and MCA-3, and Astra Pharmaceuticals for lidocaine.

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Inhibition and reversal of wheat germ agglutinin-induced caps by concanavalin A

ASSEMBLY of cell-surface components at one pole of the cell membrane, as a result of cross linkage with specific ligands, can be visualised by using labelled ligands. Such a demonstration of topographic changes on the cell surface is called capping. By using different labels for the different ligands it is possible to study the mutual relationship between various membrane components. Using the capping technique, it was possible to demonstrate the relationship between concanavalin A (con A) receptors and immunoglobulin (Ig) receptors on the lymphocyte surface[1], as well as other surface antigens such as H-2, HLA and θ antigens present on murine and human lymphocytes[2–4]. The present study was undertaken to demonstrate the relationship between the receptors of two plant lectins, con A with specificity for glucoside residues, and wheat germ agglutinin (WGA) with specificity for *N*-acetyl-glucosamine residues[5], that are both present on the surface of murine thymocytes.

Thymocytes of 4–6-week-old C57BL/6 mice were used in all experiments. Con A, WGA, con A labelled with rhodamine (Rh–con A) and WGA labelled with fluorescein (Fl–WGA) were obtained from Miles–Yeda. Succinyl–con A was prepared in our laboratory according to Gunther *et al.*[6].

Incubation of 5×10^6 thymocytes per ml with Fl–WGA, Rh–con A or with both lectins simultaneously, induced capping on the cells. Table 1 *a–d* shows the results of such experiments. It can be seen that Fl–WGA induced cap formation in 91% of the cells, while Rh–con A only in 7%. When cells were incubated with Fl–WGA and unlabelled con A or with Fl–WGA and Rh–con A, however, only 2% of the cells showed caps to WGA, whereas 89% of the cells showed a uniform distribution of the Fl–WGA around the cell surface. The presence of con A inhibited cap formation induced by Fl–WGA (Fig. 1*c*). Addition of succinyl–con A did not cause such an inhibitory effect (Table 1*e*). Pretreatment of the thymocytes with 10^{-4} M colchicine affected con A capping, as can be seen in Table 1*f–h*. This pretreatment elevated the percentage of cells with caps to Rh–con A from 7 to 22. Such pretreatment also elevated the percentage of caps to Fl–WGA from 2 to 19% in cells simultaneously incubated with con A. Moreover, the location on the cell surface of Fl–WGA caps was identical to the location of Rh–con A caps (Fig. 1*d*). The same phenomenon was also observed when Rh–con A treated cells were washed, incubated

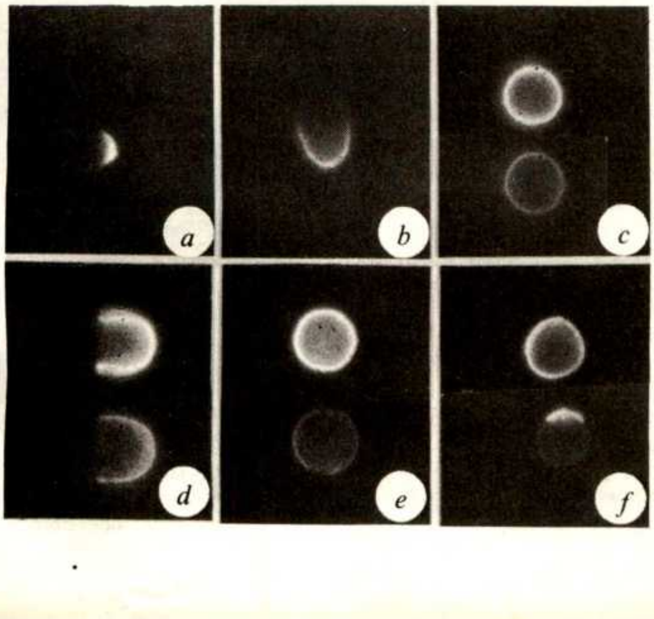
Table 1 Inhibition and cocapping of WGA receptors by con A

Treatment of thymocytes	Distribution of WGA receptors % caps	% uniform	% unlabelled	Distribution of con A receptors % caps	% uniform	% unlabelled
a Fl–WGA	91	0	9	NT	NT	NT
b Rh–con A	NT	NT	NT	7	93	0
c Fl–WGA+con A	2	89	9	NT	NT	NT
d Fl–WGA+Rh–con A	2	89	9	6	94	0
e Fl–WGA+succinyl–con A	88	2	10	NT	NT	NT
f Colchicine+Rh–con A	NT	NT	NT	22	78	0
g Colchicine+con A+Fl–WGA	19	71	10	NT	NT	NT
h Colchicine+Rh–con A+Fl–WGA	19	71	10	22	78	0
i Colchicine+Rh–con A+NaN_3+Fl–WGA	20	72	8	24	76	0

Washed C57BL/6 thymocytes (5×10^6 cells per ml) were incubated for 15 min at 37 °C, in the following conditions: *a*, 24 μg Fl–WGA; *b*, 50 μg Rh–con A; *c*, 24 μg Fl–WGA+50 μg con A; *d*, 24 μg Fl–WGA+50 μg Rh–con A; *e*, 24 μg Fl–WGA+50 μg succinyl–con A; *f*, as in *b* with pretreatment of cells with 10^{-4} M colchicine; *g*, as in *c* with pretreatment of cells with 10^{-4} M colchicine; *h*, as in *d* with pretreatment of cells with 10^{-4} M colchicine; *i*, as in *h* with incubation of cells in 10 mM NaN_3 before addition of Fl–WGA.

for 5 min in a solution containing sodium azide (non-capping conditions), and then Fl–WGA was added (Table 1*i*). These results resemble cocapping obtained with con A and anti-Ig on the surface of lymphocytes pretreated with colchicine[7]. In colchicine treated thymocytes the morphology of con A caps differs from that of WGA caps in that con A caps are elongated (Fig. 1*b*), whereas WGA caps are round (Fig. 1*a*). When cocapping occurs, however, Fl–WGA caps assume the morphology of con A caps. Addition of con A to thymocytes preincubated with Fl–WGA reversed WGA caps to a uniform distribution 30 min after the addition of con A (Table 2*b*) (Fig. 1*e*). This reversion of WGA caps was dependent on the con A concentration: addition of 50–12 μg con A ml^{-1} induced a complete reversal, while smaller amounts (6 μg and less) decreased the extent of reversal. This decrease was proportional to the amount of con A used. Such a reversion of Fl–WGA caps in thymocytes was also observed when con A was added at 4 °C, or when cells were treated with sodium azide (10 mM) before addition of con A (Table 2*d* and *e*). Thus, reversion of WGA caps by addition of con A occurs even in conditions in which cap formation is usually inhibited[8]. These results resemble the reversion of con A caps by cytochalasin B which is similarly unaffected by sodium azide[4]. Reversion of WGA caps was not observed when succinyl–con A was added 30 min after WGA-induced caps were already formed (Table 2*g*).

Fig. 1 Inhibition and reversion of caps induced by WGA. The photographs were taken using a Zeiss Universal microscope (barrier filters were used for observations of fluorescein fluorescence and rhodamine fluorescence). *a*, Cell stained with Fl–WGA at 37 °C for 15 min; *b*, cell stained with Rh–con A at 37 °C for 15 min in the presence of 10^{-4} M colchicine; *c*, cell stained with Rh–con A and Fl–WGA at 37 °C for 15 min. Both lectins were added simultaneously. Upper, Rh–con A; lower, Fl–WGA. *d*, Cocapping of Fl–WGA with Rh–con A in the presence of 10^{-4} colchicine. Upper, Rh–con A; lower, Fl–WGA. *e*, Reversion of WGA caps by con A. A cell stained with Fl–WGA, after 15 min Rh–con A was added for additional 30 min. Upper, Rh–con A; lower, Fl–WGA. *f*, Cell stained with Fl–WGA, after 15 min anti-WGA was added for 5 min, after which Rh–con A was added for additional 30 min. Upper, Rh–con A; lower, Fl–WGA.



Addition of α-methyl-D-glucoside (0.1 M) after reversion of WGA caps by con A resulted in the reappearance of WGA caps (Table 2*c*). Addition of antibodies to WGA after cap formation by this lectin, prevented the cap reversion caused by addition of con A (Table 2*f*). Moreover, when Rh–con A was added to thymocytes which had been incubated with WGA and subsequently with antibodies to WGA, it was possible to simultaneously demonstrate Fl–WGA caps and uniform distribution of Rh–con A (Table 2*f*), (Fig. 1*f*). Thus, addition of a specific cross linkage agent of WGA (anti-WGA) overcomes the antagonistic effect of con A.

The data in the present study demonstrates a mutual relationship between WGA and con A receptors on the cell surface of murine thymocytes. Receptors cross linked with con A control the topographic distribution of WGA receptors. In the presence of con A not only is WGA capping inhibited, but existing WGA caps are reversed to a uniform distribution. Such reversion is not affected by treatments involving sodium azide or 4 °C, indicating a process that does not require energy. Such a reversion in WGA caps can be overcome by addition of antibodies to

Table 2 Reversal of WGA caps by con A

Treatment of thymocytes	% WGA caps
a Fl–WGA	91
b Fl–WGA+con A	3
c Fl–WGA+con A+α-methyl-D-glucoside	91
d Fl–WGA+NaN_3+con A	2
e Fl–WGA+4 °C+con A	2
f Fl–WGA+anti-WGA+con A	89
g Fl–WGA+succinyl–con A	88

The cells were not washed after Fl–WGA treatment, but identical results were obtained when cells were washed after this primary incubation. In experiment 2*a* 5×10^6 thymocytes per ml were incubated at 37 °C for 15 min in the presence of 24 μg Fl–WGA. In experiment 2*b* 5×10^6 thymocytes per ml were preincubated at 37 °C for 15 min in the presence of 24 μg Fl–WGA and then incubated with 50 μg con A at 37 °C for 30 min. In experiment 2*c* 5×10^6 thymocytes per ml were preincubated at 37 °C for 15 min in the presence of 24 μg Fl–WGA, then incubated with 50 μg con A at 37 °C, after 30 min α-methyl-D-glucoside (0.1 M) was added at 37 °C for 15 min. In experiment 2*d–f*, 5×10^6 thymocytes per ml were preincubated at 37 °C for 15 min in the presence of 24 μg Fl–WGA. Then the first sample was incubated with NaN_3 (10 mM) at 37 °C. The second at 4 °C. The third—with anti-WGA (5 μg Ab ml)—at 37 °C. After 5 min 50 μg con A was added to the 3 samples for 30 min at the above-mentioned temperatures. In experiment 2*g*, 5×10^6 thymocytes per ml were preincubated at 37 °C for 15 min in the presence of 24 μg Fl–WGA and then incubated with 50 μg of succinyl–con A for 30 min at 37 °C.

WGA, which probably increases the extent of cross linkage of WGA receptors.

Our results correlate with previously published studies dealing with the effect of con A on the capping of mouse lymphocyte surface immunoglobulin and on the capping of mouse thymocyte antigens[1,4]. Our results could be interpreted by assuming that the surface molecule carrying con A receptors also carries WGA receptors as suggested for the case of con A and Ig receptors[1]. This explanation is, however, not feasible for WGA and con A receptors since, in the presence of anti-WGA serum, WGA causes capping while con A does not. The WGA used in the present study is completely free of contaminating sugars, as assayed by the phenol–sulphuric acid test[9], which precludes the possibility that con A binds to contaminated sugar residues present in the WGA preparation. Our data suggest the following alternative explanation: it is known that con A receptors of thymocytes and lymphocytes are a heterogeneous population in valence, affinity and chemical composition[10] and are also known to be bound to microtubules which restrict their mobility[4]. In addition, the con A molecule is tetravalent[11] while WGA is divalent[12], thus enabling con A to form a firm lattice-network on the surface of the thymocytes. This con A receptor lattice may trap WGA receptors as well as other receptors such as Ig and θ antigens which also seem to be interspersed among con A receptors[4]. As a consequence the mobility of WGA receptors is governed by the lattice formed by con A, and therefore the WGA caps can be reversed by the denser lattice formed by con A when binding to its receptors.

Our explanation of the cap-reversion phenomenon is supported by the following two observations: (1) substitution of tetravalent con A with divalent succinyl–con A does not cause a reversion of previously formed WGA caps, and (2) addition of antibodies to WGA prevents con A reversion of WGA caps, apparently by increasing the extent of cross linkage of WGA receptors. Preliminary results in our laboratory have shown that the reversion phenomenon also occurs with Ig receptors by con A.

We thank Mrs O. Asher for technical assistance. This study was supported partly by a grant from Bar-Ilan University.

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Received November 3, 1975; accepted January 5, 1976.

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Evidence for a soluble lymphocyte factor in the transplacental transmission of T-lymphocyte responses to *Mycobacterium leprae*

ANTIGENS introduced to the human foetus have been considered as "self" and thus not to provoke an immune response[1]. But, no sudden changes in the immune mechanisms seem to coincide with birth, and the development of adult immune responses is gradual. Moreover, in sheep, antigens can give rise to more or less mature immune responses in the foetus[2,3], dependent on the stage of gestation. The nature of transmission of these immune responses from mother to foetus remains unclear. Brody *et al.*[4] demonstrated in humans that T-lymphocyte sensitisation could be transmitted from mother to foetus and considered this was due to transplacental passage of antigen. Field and Caspary[5] showed that cell-mediated responses can be induced in the foetus, but thought that either maternal lymphocytes or some subcellular lymphocyte factor crossed the placenta to sensitise the foetal lymphocytes. We have tested ten randomly selected normal mothers and their babies at parturition for evidence of sensitisation to antigens of *Mycobacterium leprae*. We found that when the mothers showed sensitisation their babies did too: the children of unsensitised mothers were not sensitised. We consider the likeliest explanation for our findings is the transplacental passage of a soluble lymphocyte factor.

Lymphocyte transformation tests (LTT) were carried out by a micromethod[6] on both maternal and neonatal cord blood; whole washed *M. leprae* were used as antigen, and in each case the responses to BCG and PPD were also determined. Of the ten mothers tested, five showed a positive LTT response with *M. leprae* as antigen and five did not (Fig. 1). It was considered that a positive response existed when the LTT response with the antigen was more than

Fig. 1 LTT responses to whole *M. leprae* in mothers and neonates, comparing the five mothers with positive responses and the five mothers with negative responses (unstimulated control values are subtracted). ^{3}H-TdR, Tritiated thymidine uptake.

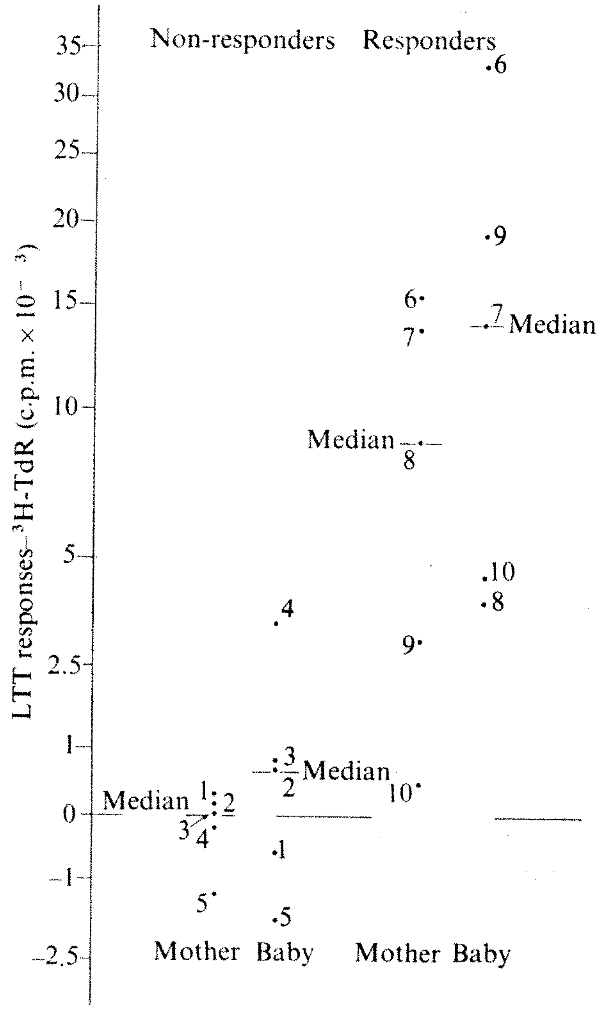

Table 1 Median responses to antigens

	M. leprae		BCG		PPD	
Mothers with positive responses to *M. leprae*	8,600	$P<0.005$	267	$P>0.1$	4,316	$P<0.02$
Mothers with negative responses to *M. leprae*	−11		21		−36	
Babies of mothers with positive responses to *M. leprae*	13,658	$P<0.005$	995	$P>0.1$	3,654	$P>0.1$
Babies of mothers with negative responses to *M. leprae*	602		−17		1,140	

Figures are c.p.m.

Median responses to antigens in the two maternal and the two neonatal groups. P, Probability of distribution between the two groups occurring by chance, calculated by Mann–Whitney U test.

twice the value of unstimulated control cultures. The values for these control cultures were much higher in the neonates (median: 13,171) than in the mothers (median: 532). In the five mothers with positive lymphocyte blastogenic responses, the response of the neonate reflected the maternal response, though the former were much greater. In the five unresponsive mothers, the neonatal response was much lower than in the neonates born of responders. The difference between the two neonatal groups is highly significant ($P<0.005$), using the Mann–Whitney U test for statistical analysis. There was also a statistically significant difference in responses to PPD (but not BCG) between the maternal responders and non-responders to *M. leprae* (Table 1), possibly due to some cross reactivity. There was, however, no significant difference of responses to PPD and BCG between the two neonatal groups.

Five out of ten may seem a high proportion of mothers to show evidence of sensitisation to *M. leprae;* but it is in accord with our experience that up to 90% of contacts with known cases of leprosy show evidence of previous subclinical infection[7]. Moreover, in Sao Paulo, Brazil, where the leprosy endemicity is similar to that of Addis Ababa, more than 50% of the normal population show positive lepromin (Fernandez) skin tests[8].

This study provides evidence that when mothers are sensitised to *M. leprae* this sensitisation is transmitted to the foetus. The sensitisation seems to be specific, as there was no significant difference between the responses of the two neonatal groups when BCG or PPD were used as antigens.

For transmission of this sensitisation, there are three main possibilities. The first is transplacental passage of antigen. In our study, this seems unlikely as the mothers showed no signs of leprosy, or other mycobacterial disease. The second possibility is transplacental passage of maternal lymphocytes. It is still debatable whether maternal lymphocytes enter the foetal circulation[9–12], so if they do cross the placenta it must be in very small numbers. In view of the high neonatal LTT responses, recruitment of neonatal lymphocytes by maternal lymphocytes would again seem improbable. Third, there could be transplacental passage of a soluble lymphocyte factor. It seems most likely that such a factor was responsible for the foetal lymphocyte sensitisation we have demonstrated.

The nature of this lymphocyte factor remains unclear. Some immunoglobulins are known to cross the placenta, but none has been shown to raise antigen-specific T-lymphocyte responses in unsensitised individuals. Several soluble factors for specific T-cell stimulation however, have now been described. One possibility in this context is transfer factor[13] which can transfer delayed type hypersensitivity and, having a molecular weight of less than 10,000, could cross the placenta passively. The nature of the factor suggested by our study clearly needs further investigation.

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Received November 21, 1975; accepted January 28, 1976.

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Cytoplasmic exclusion as a basis for asymmetric nucleocytoplasmic solute distributions

THE nuclear and cytoplasmic concentrations of solutes in cells are often unequal. This has been observed for ions[1,2], small molecules[3–5], and for macromolecules[6–10]. The role of maintaining these concentration differentials is commonly assigned to the nuclear envelope, but this practice is predicated on a questionable analogy with the plasma membrane. Most of the information derived from tracer kinetics[4,8,9] and electrical resistance[10–13] indicates that the nuclear envelope is too permeable to distinguish or differentially transport small solutes. Thus, we must look to other, presumably equilibrium, mechanisms to account for many nucleocytoplasmic solute asymmetries. One useful step in the analysis of solute distributions between two phases is to establish a reference phase to which the concentrations of substances in the other two phases can be compared. A suitable reference phase for cytoplasm and nucleus cannot, of course, be separated from them by the cell membrane, which itself has complex, asymmetric transport properties. Thus, the reference phase must be intracellular. We have carried out experiments in which a defined aqueous reference phase (a gelatin gel) was introduced into the cytoplasm, and the partition of solutes between this phase, cytoplasm, and nucleus was determined. We deal here with the intracellular distribution of ^{3}H-sucrose—a small, non-metabolised substance which reaches a greater concentration in the nucleus than in the cytoplasm[4].

The experimental design was as follows: a solution of 11% fluorescein isothiocyanate-labelled gelatin was microinjected into the vegetal hemispheres of grown *Rana pipiens* oocytes at 34 °C. The oocytes were immediately cooled in ice-cold Ringer's to gel the gelatin, and then brought to 15 °C. The gelatin remained gelled at this temperature and provided the intracellular reference phase. Shortly before or after gelatin injections (the sequence did not influence the results). ^{3}H-sucrose was microinjected into the oocyte cytoplasm. The oocytes were incubated for at least 3 h (adequate time for ^{3}H-sucrose to achieve diffusional equilibrium[4]), quenched to liquid nitrogen temperature, sectioned at −50 °C, and the intracellular distribution of tracer determined quantitatively by ultra-low temperature autoradiography[14]. Figure 1 is a diagram of a typical autoradiographic section showing the nucleus and the gelatin within the cytoplasm, with the local grain densities superimposed.

Silver grains were uniformly distributed under the cytoplasm, confirming that the injected ^{3}H-sucrose had reached diffusional equilibrium. Grain densities under the nucleus and gelatin were also uniform but were higher than under the cytoplasm—2.66 and 2.67 times greater, respectively. When the nucleus–cytoplasm (N–C) grain density ratio is corrected for the different water content of the two compartments (N–C = 1.88, ref. 1), a ^{3}H-sucrose partition coefficient of 1.41 is obtained for nucleus–cytoplasm. The water content of the intracellular gelatin gel phase is assumed to be that of the gelatin sol injected (89%), and the corrected ^{3}H-sucrose partition coefficient for gelatin–cytoplasm is 1.35. (It is possible that the water content of the gelatin changed subsequent to injection. Even total replacement of the gelatin by water, however, would yield a coefficient no less than 1.20). Table 1 summarises our results.

Control data include nucleocytoplasmic partition coefficients in oocytes injected only with ^{3}H-sucrose, and the dialysis partition coefficient between 11% gelatin and buffer. These controls show that intracellular gelatin does not affect the nucleocytoplasmic partition of ^{3}H-sucrose. Furthermore, the ^{3}H-sucrose asymmetries cannot be attributed to affinities of either gelatin or nucleus for ^{3}H-sucrose, since the gelatin–buffer concentration ratio is essentially unity, as is that of nucleus–gelatin. We conclude that there must be an exclusion of ^{3}H-sucrose from some of the cytoplasmic water—that is, cytoplasmic water is less available as a solvent than the water of gelatin, the nucleus, or buffer. The data may be explained if 4% of the nuclear water and 32% of the cytoplasmic water are unavailable as solvent for ^{3}H-sucrose.

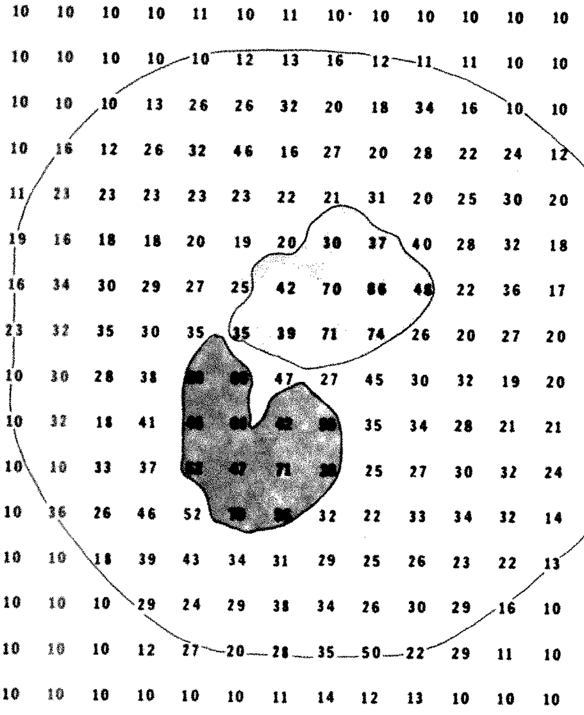

Fig. 1 Diagram of an ultra-low temperature autoradiographic section through the oocyte nucleus (light shading) and gelatin (dark shading). A volume of 11% fluorescein isothiocyanate-labelled gelatin about equal to that of the nucleus was microinjected as a sol (at 34 °C) in buffer (15 mM NaCl and 130 mM K phosphate, *p*H 7.1). After gelation (at 0 °C) of the gelatin, 0.2 μCi ^{3}H-sucrose (New England Nuclear) in 5×10^{-5} ml water was microinjected and the oocyte incubated at 15 °C for a time sufficient for the ^{3}H-sucrose to reach diffusional equilibrium (3–4 h). The oocyte was then rapidly embedded in OCT and quenched to −190 °C in liquid nitrogen, with sectioning and autoradiographic exposure carried out at ultra-low temperatures to prevent ^{3}H-sucrose redistribution[14]. The nucleus was identified by its size, location in the animal hemisphere, absence of yolk platelets and pigment granules, and the presence of chromatin and nucleolar clumps. The gelatin appeared as an inclusion-free area which was positively identified by its fluorescence in blue light illumination. Superimposed on the section are relative local grain densities, determined photometrically with a Leitz MPV-1 microscope photometer using Ploem incident light illumination[22]. The grain density values shown are absolute photometer readings, and have not been corrected for the different water contents of the cytoplasmic, nuclear and gelatin compartments. The data summarised in Table 1 were derived from 18 similar two-dimensional grain-density plots from sections taken from five cells.

Table 1 Equilibrium partition of ^{3}H-sucrose between aqueous phases at 15 °C

Phases	K*
Gelatin-injected cells	
Nucleus–cytoplasm	1.41 ± 0.03
11% Gelatin–cytoplasm	1.35 ± 0.03
Nucleus–11% gelatin	1.04
Controls	
Nucleus–cytoplasm (no gelatin injected)	1.34 ± 0.08
11% Gelatin–buffer (dialysis chamber)	0.98

* Ratio of ^{3}H-sucrose concentrations in the water of the respective phases at diffusional equilibrium (± s.e.m.).

Nucleocytoplasmic concentration asymmetries in oocytes have been previously reported for glycerol[3], unbound Na$^+$ (ref. 2), α-aminoisobutyric acid[5], inulin[8], three molecular sizes of dextran[9], and exogenous proteins[7,15]. Also, endogenous oocyte proteins exhibit nucleocytoplasmic distributions greater than unity[15,16], although their distributions may be caused in part by mechanisms other than cytoplasmic exclusion (for example, specific binding to nuclear components or asymmetric nuclear envelope transport).

Many other types of cells show asymmetric nucleocytoplasmic distributions of solutes, but without experimental analysis of the type described here, cases of cytoplasmic exclusion cannot be distinguished from asymmetries caused by other mechanisms. Nevertheless, because of the nature of the solutes and the magnitudes of the asymmetries observed, we suggest that the intracellular distributions of colloidal gold in amoeba[18,19] and of fluorescein-labelled exogenous proteins in salivary gland cells[10] of *Chironomus* may reflect the operation of cytoplasmic exclusion. It is likely that the asymmetric nucleocytoplasmic equilibrium distribution of the shuttling proteins of amoeba[17] is also explicable by cytoplasmic exclusion processes. Data from these diverse cell types suggest that the concentration of soluble cellular components in the nucleus occurs by cytoplasmic exclusion in many cells.

Intracellular concentration differences are not restricted to the nucleus, and other organelle–cytoplasm solute distributions may reflect the operation of cytoplasmic exclusion. Specifically, cytoplasmic exclusion may have a previously unexpected role in determining solute distributions between the cytoplasm, vacuoles, and the cisternae of the endoplasmic reticulum and the Golgi apparatus.

Our observations demonstrate the existence of cytoplasmic exclusion: they do not specify the molecular mechanism(s) responsible. It is clear, however, that much less cytoplasmic water is available as solvent than is measurable by subtraction of dry from wet weights. The operative mechanisms may include changes in bulk solvent properties of water through electrostriction, hydrophobic interactions, and solvent–polymer H bonding, and/or restriction of the accessibility of the water to solute through solvent microcompartmentalisation or polymer–solute repulsive interactions. Analogues for the behaviour of the cytoplasm are found in other macromolecular systems, such as solutions of

441.6–400 nm, reaching a value of less than 0 02 at the vaccinia virus[20], hyaluronic acid[21], and the cross-linked dextrans used in chromatography.

Whether these or other mechanisms are responsible for the exclusion behaviour of cytoplasm, the process itself should not be overlooked in seeking the explanation for asymmetric distributions of solutes between the nucleus and cytoplasm.

We thank Drs C. Feldherr, K. Magleby, P. Furmanski and B. Rose for criticism. This work was supported by the NIH and an institutional grant to the Michigan Cancer Foundation from the United Fund of Greater Detroit.

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Retinal sensitivity to damage from short wavelength light

A GROWING body of literature attests to the deleterious effects of long term exposure to light[1–8]. To define more critically the differences between thermal and photochemical effects, we have exposed the retinae of rhesus monkeys to eight monochromatic laser lines from 1,064–441.6 nm. Thermal damage to the retina is to be expected for the 1,064-nm line since the photopigments are not involved and energy absorption takes place predominantly in the melanin granules of the pigment epithelium and the choroid Although data on pathogenesis are not yet available, we found some interesting differences in retinal sensitivity in going from the near infrared to the blue wavelengths in the visible spectrum

In a recent paper[9] on solar retinitis in the rhesus monkey, we pointed out that a simulated solar exposure of 3 min duration produced a retinal lesion which could not be explained solely in terms of thermal injury, but which must cause some damage by a photochemical mechanism. We postulated some type of thermally enhanced photochemical effect similar to Noell's findings[1]. It now seems possible to account for solar retinitis almost entirely in terms of the photochemical effects of the short wavelength components in the Sun's spectrum Tso[10] has reported on some patients who gazed voluntarily at the Sun for an hour before submitting to enucleation for malignant melanoma. An estimate of the maximum temperature on the retina using White's[11] mathematical model for solar observation yields approximately 1.5 °C above ambient for a 2-mm pupil. Whereas these patients suffered minimal visual acuity effects and obviously did not sustain retinal burns, the pathology after enucleation indicated damage to the retina which must have resulted from photochemical processes aided perhaps by thermal enhancement.

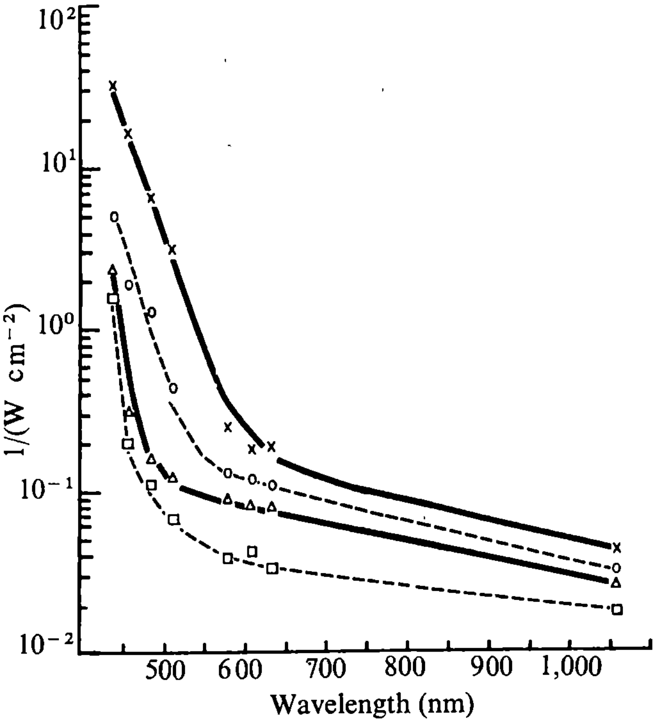

Fig. 1 Action spectrum of retinal sensitivity to threshold damage in the rhesus monkey. Reciprocal of retinal irradiance ($1/\text{W cm}^{-2}$) is plotted semi-logarithmically against wavelength (nm) for exposure durations of 1, 16, 100 and 1,000 s. Beam diameter on the retina was 500 µm to the $1/e^2$ points of the Gaussian distribution. □, 1s; △, 16 s; 0, 100 s; ×, 1,000 s.

Results of the present experiments are summarised in Table 1, in which maximum retinal irradiance (W cm^{-2}) and maximum temperature in the retina above ambient (°C) are given for each exposure duration and wavelength. Maximum irradiance H_0 is based on the Gaussian distribution of the laser beam

$$H = H_0 \exp(-r^2/2\sigma^2)$$

where H is the irradiance at radius r from the Gaussian peak and σ is calculated from the beam profile by measuring r at the $1/e^2$ points. H_0 on the retina is calculated from the formula

$$H_0 = P_c T / 2\pi\sigma^2$$

where P_c is the power (W) entering the cornea and T the transmittance through the ocular media of the rhesus monkey as measured in our laboratory on nine eyes from five animals. An irradiance of 24 W cm^{-2} at a temperature of approximately 23 °C above ambient produced a threshold lesion in 1,000 s with the Nd-YAG laser (1,064 nm) In contrast, the 441 6-nm line from the He:Cd laser required only 30 mW cm^{-2} with negligible temperature rise to produce a threshold lesion in a 1,000-s exposure. The ratio of the two irradiances on the retina is 800. The Nd–YAG lesion has the typical appearance of a burn lesion, whereas the He–Cd lesion has no central core but appears as a slight patch of yellowish-white on the fundus, often with irregular boundaries. We attribute the lesion produced by 1,064-nm radiation to thermal effects, whereas that produced by the 441.6-nm line must be caused by photochemical processes.

Figure 1 is an action spectrum obtained by plotting semi-logarithmically the reciprocal of retinal irradiance ($1/\text{W cm}^{-2}$) against wavelength (nm) for each exposure duration The increase in retinal sensitivity with decrease in wavelength is steep even on a semi-logarithmic plot and shows no tendency to peak at 441.6 nm. Transmittance through the ocular media falls rapidly for wavelengths 441 6–400 nm, reaching a value of less than 0.02 at the

Table 1 Retinal irradiance H_0 (W cm^{-2})±s.d. for threshold lesion as function of wavelength (nm) and exposure duration (s)

Laser	Wavelength (nm)	Exposure duration (s)				Transmittance (*T*)
		1	16	100	1,000	
Nd–YAG	1,064	56.1±5.3 55°	37.5±4.2 37°	32.5±3.1 32°	24.0±3.0 23°	0.76
He–Ne	632.8	29.9±1.4 49°	15.2±3.0 25°	8.4±2.8 14°	5.4±2.3 9°	0.93
Ar-pumped dye laser	610	22.0±1.9 36°	12.3±1.1 20°	8.1±1.1 13°	5.8±0.3 9.5°	0.92
	580	26.1±4.5 43°	11.5±2.4 19°	7.6±0.8 12.5°	4.0±1.7 6.6°	0.91
Ar	514.5	14.5±3.3 25°	10.3±2.3 18°	2.2±0.5 4°	0.32±0.1 1°	0.87
Ar	488	9.4±1.2 17°	6.1±0.8 11°	0.77±0.2 1°	0.15±.08 0.25°	0.83
Ar	457.8	5.1±0.8 10°	3.2±0.6 6°	0.52±0.1 1°	0.06±.02 0.1°	0.69
He–Cd	441.6	0.91±.03 2°	0.41±.02 1°	0.20±.02 0.4°	0.03±.01 0.05°	0.45

Maximum retinal temperature (°C) above ambient estimated from mathematical model of Clarke[12]. *T*, Transmittance through ocular media. Beam diameter on retina 500 μm to $1/e^2$ points. Entire laser beam, TEM$_{00}$ mode, enters eye of anaesthetised animal with pupil dilated to 8 mm. A beam splitter and fundus camera are used to view and direct beam on the retina. Criterion for a threshold lesion is the appearance 24 h after exposure of a funduscopically visible lesion. For details of method of determining thresholds, see ref. 9.

lower wavelength. This is caused primarily by absorption in the lens. Whereas the sensitivity of the retina may continue to increase in the wavelength band 441–400 nm, the number of photons reaching the retina is reduced drastically by absorption and scattering in the lens and the rest of the ocular media. In fact, the threshold for damage may shift from the retina to cataractogenesis in the lens. We are attempting to explore this region of the spectrum using narrow bandpass filters with a 2,500-W xenon arc lamp. Also, it is planned to expose the retina of the monkey after surgical removal of the lens.

These results help to explain the phenomenon of solar retinitis which cannot be interpreted solely in terms of thermal injury. Unattenuated sunlight peaks at 470 nm and a relatively large proportion of the solar energy is concentrated in the blue end of the spectrum. For example, the solar retinal irradiance at 440 nm for a 20-nm spectral band is approximately 0.20 W cm^{-2} at midday for an eye gazing directly at the Sun at sea level with a 2-mm pupil. In comparison, the threshold irradiance for a 100-s exposure to the 441-nm laser line of He–Cd is 0.20 W cm^{-2}. Thus, sungazing at bright midnoon for 100 s can produce a threshold lesion. It is our belief that solar retinitis is caused primarily by the short wavelengths in the visible spectrum of the Sun, with some thermal enhancement from the longer wavelengths in the visible and near infrared. The retinal pathology among military personnel on a Pacific island as reported by Smith[13] and the aetiology of foveomacular retinitis among military personnel as reported by Marlor[14] can be better understood in terms of these results. Those subject to exposure to bright sunlight over long periods should take precautions to shield their eyes from the short wavelengths of solar radiation. This has implications in the design of sunglasses and welders' goggles. The sensitivity of the retina to short wavelengths of light may also have clinical significance for certain retinal pathologies such as senile macular degeneration and retinitis pigmentosa.

The sensitivity of the retina to blue light may have more profound effects for man-made optical sources than for sunlight. The trend towards ever brighter sources in the blue end of the spectrum—high pressure Hg and xenon arc lamps, and the 'natural' lighting sources in current use—are examples. The ocular hazards associated with lasers may require re-evaluation. Arc welding is another example where the eyes may need special protection from blue light. Children undergoing phototherapy for bilirubinemia should have their eyes protected and the growing population of elderly people with aphakic eyes will need protection from the short wavelengths in the visible and near ultraviolet spectrum.

The basic photochemical mechanisms underlying the sensitivity of the retina to short wavelengths of visible light are unknown. Our preliminary results indicate that the lesion is located in the outer segment of the photoreceptor and perhaps in the pigment epithelium. We have not as yet been able to distinguish between rod and cone damage and do not know to what extent the photopigments are involved. Most previously reported long term photic damage results from exposure to bright environmental lighting over periods of days or weeks, whereas our exposures extend from 1–1,000 s and are confined to an extremely small and finite portion of the retina. Anderson *et al.*[15] have reported similar sensitivity to blue light in albino rats in environmental lighting conditions. A number of mechanisms to explain long term photic damage to the retina have been proposed. These include photo-oxidative membrane damage, toxic products in the outer retina, metabolic disorders from extended overbleach, and so on. In our opinion the continued increase in sensitivity with decreasing wavelength indicates that additional molecular systems become vulnerable to electronic excitation and photic damage as the energy per photon increases. This is to be expected and we believe that several photochemical mechanisms are probably responsible for the observed damage. We do not expect the action spectrum to peak as the near ultraviolet is approached.

Drs R. Peterson and R. James, Laser Section, Division of Electronic Products, Bureau of Radiological Health, provided exposures in their laboratory to the Nd–YAG 1,064-nm laser radiation. Supported jointly by the Bureau of Radiological Health, the Food and Drug Administration, the US Army Medical Research and Development Command, and the General Electric Foundation. In conducting the research described in this report, the investigators adhered to the *Guide for Laboratory Animals Facilities and Care* of the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

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Visual adaptation produced by gratings with no brightness contrast

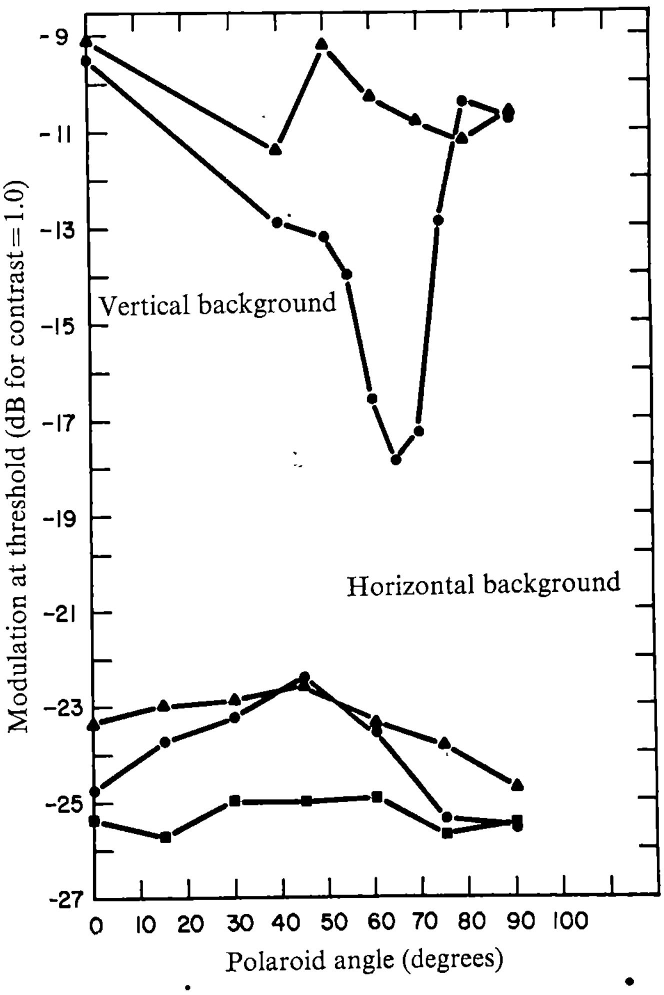
A STRIPED grating is more difficult to see after prolonged viewing of a similar grating[1,2]. Similarly, the contrast threshold is elevated when a test grating is briefly presented superimposed on a continuously viewed grating[3,4]. Here we show that the threshold is raised by gratings that have no brightness difference between alternate stripes, but only a hue difference. We measured luminance modulation thresholds for seeing a yellow grating superimposed on a background of interdigitated red and green stripes. We varied the relative luminances of the red and green stripes while holding the sum of their luminances constant. Thus we could be certain that for some luminance ratio, the red and green stripes must have had the same subjective or effective brightness for the observer. Nevertheless, we found that for all ratios of red-to-green luminance the contrast threshold for the test grating was higher than that obtained in several control conditions (including backgrounds with the same colours and configuration but perpendicular to the test grating, and uniform backgrounds with the same mean spectral composition).

Yellow test gratings were produced by conventional techniques on an oscilloscope with P31 phosphor, viewed at a distance of 3 m through an Ilford 201 filter. The test field was a 13 cycles per degree sine grating, 2° square. The background gratings, also 13 cycles per degree, were produced by two projectors whose beams fell on a ground glass screen after being merged by a half-reflecting pellicle. Each projector cast an image of a square-wave grating on the screen. An Ilford 624 (bright spectrum green) filter was placed in the beam of one projector, and an Ilford 205 (narrow cut tricolour red) filter in the beam of the other. Polaroid filters oriented perpendicularly with respect to each other were also interposed in the separate beams. The relative intensities of the red and green components could then be varied by rotating a Polaroid interposed in the common beam. The positions of the red and green gratings could be adjusted so as to superimpose the red and green stripes or to interdigitate them. When red and green were superimposed, the grating varied in appearance from red-and-black through yellow-and-black to green-and-black as the Polaroid in the common path was rotated. When red and green were interdigitated, the appearance varied from red–black through red–green to black–green. The screen was viewed from the side opposite the projectors at a distance of 3 m, and was seen through a pellicle that merged its image with that of the test grating. A dove prism close to this pellicle, but only in the path to the background screen, controlled the orientation of the background grating. Various adjustments allowed for the precise control of orientation, phase, and magnification of test and background gratings. The mean luminances over the area at the observer's eye were 6.2 and 3.9 cd m^{-2} for the red and green components at their respective maximum settings and 6.2 cd m^{-2} for the test field.

Contrast thresholds were measured by a two-staircase up-and-down procedure. When the observer pressed a key the test screen was changed from homogeneous to striped for 100 ms. If he saw the test grating, its contrast was reduced by 1 dB (about 12%) on the next trial on that staircase. If he did not see it, it was raised by 1 dB. Random selection of the staircase operative on each trial reduced the observer's ability to predict the changes in contrast from trial to trial. All of the results are contrast thresholds for detection of a yellow vertical sine grating; therefore any differences in thresholds are due to the differences in the backgrounds on which the test grating was presented.

Figure 1 plots the contrast thresholds for the test grating on various backgrounds. The x axis represents the relative luminances of the red and the green background stripes, as determined by the setting of the variable Polaroid. Thresholds were nearly independent of Polaroid angle for all background conditions except vertical interdigitated red–green gratings. With interdigitated vertical gratings, however, the threshold was found to vary with relative luminance of the red and green stripes, reaching a minimum at a Polaroid angle of about 65°. Even at this point, though, the threshold was elevated substantially—5 dB higher than in any of the three control

Fig. 1 Contrast thresholds for a vertical yellow test grating (13 cycles per degree) presented briefly against various backgrounds: interdigitated red and green stripes, superimposed red and green stripes, uniform yellow field. Relative luminance of red and green components was varied by rotating a Polaroid filter in the dual optical system. For interdigitated backgrounds Polaroid settings of 0° produced red–black and 90° black–green gratings respectively. All data are from the same observer. Mean s d. of observations, 0.94 dB. ●, Interdigitated stripes; ▲, superimposed stripes; ■, uniform background.



conditions (horizontal interdigitated, horizontal superimposed stripes, or uniform backgrounds of the same mean luminance and mean spectral composition as the striped fields). Essentially the same result has been obtained in several repetitions of this experiment using other grating frequencies (3 and 6 cycles per degree) and a second observer. We have always found some threshold elevation (at least 5 dB) with any luminance ratio of the red–green stripes. Thus it seems reasonable to conclude that threshold elevation can occur without luminous contrast in the adapting stimulus.

In this experiment the more intense stripes of the yellow test grating were aligned with the green stripes of the interdigitated backgrounds. An auxiliary experiment tested whether the relative position of background and test gratings had any effect on the contrast thresholds. Phase was varied in 30° steps from the condition where the more intense yellow steps were superimposed on the green background stripes to that where they were superimposed on the red background stripes. There was no systematic effect of phase. Threshold was 6 dB higher than the control condition with a maximum deviation of any point of 1 dB from the mean. Thus the variation of contrast thresholds with Polaroid angle (Fig. 1) cannot be attributed to phase effects.

Although thresholds are never as low on red–green vertical backgrounds as they are on the various control backgrounds, they do reach a minimum for an intermediate setting of the Polaroid, that is, when the luminance contrast is small or nonexistent. As shown in Fig. 1, this does not happen when the red and green stripes are in phase.

Two interpretations of the minimum in the curve with red–green interdigitated stripes are plausible. On the one hand, adopting a form of opponent-colour theory, it may be supposed that pattern information is carried by both luminance and chromatic channels. When the Polaroids were set at their extreme positions, producing red–black or green–black gratings, both classes of channels were activated, but in the middle range only the chromatic channels received inputs, thus resulting in less elevation of the threshold. Alternatively, it might be held that the sum of the contrasts in the primary cone mechanisms determines the degree of threshold elevation. Since the red light used stimulates the green cone mechanism (π_4) as well as the red cone mechanism (π_5) and the green light π_5 as well as π_4, the sum of the contrast in these mechanisms is greatest at the extreme settings of the Polaroid and reaches a minimum for some intermediate setting. It is not possible with the available data to choose between these alternative hypotheses.

From a simple, subjective point of view, it is perhaps not surprising that equally bright red and green stripes elevate the threshold for seeing gratings, since they are visible as gratings. Perhaps the threshold elevation is simply related to the visibility of the adapting pattern.

We thank F. W. Campbell in whose laboratory these experiments were conducted and C. Hood for assistance. J.K. was a Visiting Scholar at St John's College, Cambridge University, and held a US Public Health Services Special Postdoctoral Fellowship.

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Dissociation of response to injected gonadotropin between the Graafian follicle and oocyte in pigs

THE process of ovulation in mammals is induced by a surge of gonadotropic hormone(s) that precipitates a series of changes in the cellular layers of the Graafian follicle. The changes in the somatic cells are extensive, ranging from enzymatic transformation in the biosynthetic pathway for steroid hormones[1,2] to morphological alterations in the membrana granulosa. In contrast, the only conspicuous modification in the germ cell is completion of the first meiotic division with extrusion of a polar body and formation of the second metaphase plate. The meiotic steps from diakinesis to first polar body formation are known to be programmed with considerable accuracy[3,4] by the endogenous surge of luteinising hormone (LH), but a similar sequence of nuclear changes can also be initiated by injection of a preparation rich in LH activity, such as human chorionic gonadotropin (HCG)[5–7]. Although the changes in the somatic cells and oocyte within the mature follicle usually occur in concert, we describe here circumstances in which the process of ovulation is dissociated from nuclear maturation of the germ cell, resulting in the shedding of a primary oocyte.

Mature Large White gilts with 20- or 21-d oestrous cycles were assigned to one of four groups and given a single intramuscular injection of 500 or 1,000 IU of HCG (Organon) in saline on day 17, 18, 19 or 20 of the cycle. Ovulation occurs 40–42 h after such an injection given during prooestrus[8,9]. Animals were tested immediately before injection with a mature boar to ensure that they were not in oestrus, a procedure continued twice daily until slaughter. Between 24 and 30 h after injection of HCG, laparotomy was performed under Nembutal–halothane anaesthesia and 5 ml of semen containing $1.99–2.74 \times 10^8$ spermatozoa per ml was instilled into each uterine horn. Animals were slaughtered 44–72 h after receiving HCG, and ova were flushed from the Fallopian tubes with Eagle's medium. These were fixed in acetic alcohol, stained with orcein and examined by phase contrast microscopy.

In 15 control animals, the steroid hormone environment of the oocyte was estimated in mid-follicular phase and shortly before ovulation. Follicular fluid was obtained at laparotomy during occlusion of the ovarian blood supply or immediately on ovariectomy, by aspiration into 1-ml plastic syringes through a 26-gauge needle Follicular fluid was stored at −20 °C until it could be analysed for oestradiol by radioimmunoassay; the method was essentially that of Hotchkiss *et al.*[10] but used an antiserum raised in sheep against 6-oxoestradiol coupled through a carboxymethyloxime bridge to bovine serum albumin. Progesterone was estimated by radioimmunoassay[11] using an antiserum raised in sheep against 11α-hydroxyprogesterone coupled through a succinate bridge to bovine serum albumin. Follicular fluids from animals injected with HCG were also examined by radioimmunoassay for LH activity.

Table 1 summarises the number of animals injected; the responses can be considered in terms of the maturation of several systems Display of behavioural oestrus was exceptional after HCG injection on day 17 or 18, but it was a predictable sequel to treatment in the later part of the follicular phase Likewise, after injection on day 17, the ovarian response to HCG was usually superovulation, whereas the mean ovulation rates after an injection on day 18, 19 or 20 did not differ significantly from those arising spontaneously in comparable animals.

The nuclear maturity of oocytes recovered from the Fallopian tubes was also related to the time of HCG injec-

Table 1 Responses to HCG

	System under consideration	Behavioural oestrus		Ovarian response		Maturity of oocyte nucleus	Block to polyspermy	Availability of vitelline factors (MPGF)†
Day of oestrous cycle* when HCG injected	No. of animals injected	Animals showing oestrus No.	(%)	Recent ovulations Total no.	Mean per animal	Primary oocytes (%)	Polyspermic eggs (%)	Eggs containing transformed sperm heads (%)
17	7	0	0	130	18.6	18.2	91.7	<10
18	9	1	11.1	112	12.4	17.4	38.0	
19	45	39	86.7	581	12.9	2.1	2.4	
20	27	25	92.6	308	11.4	1.4	2.8	>90

Figures show results obtained from 88 post-puberal pigs illustrating five aspects of the response to a single intramuscular injection of HCG given at various stages of the follicular phase of the oestrous cycle. These results can be considered in terms of a series of biological systems which undergo maturation during the latter part of the oestrous cycle.

*Only animals with oestrous cycles lasting 20 or 21 d were assigned to this experiment.

†MPGF, Male pronucleus growth factor[14].

tion: most oocytes recovered after day 17 treatment possessed a dictyate nucleus in which the chromatin was condensed around a single large nucleolus. Such primary oocytes were tightly encompassed by cumulus cells. By contrast, after HCG injection on day 199 or 20, recovery of primary oocytes was rare, only 14 being observed among 785 ova examined from these groups.

Maturation of the mechanism underlying the block to polyspermy was also inferred to be taking place during the follicular phase, since 92% of the oocytes recovered after treatment on day 17 exhibited polyspermy compared with 2–3% after treatment on day 19 or 20 (Table 1). Moreover, the extent of the polyspermic condition in individual primary oocytes was frequently massive, with as many as 60–80 unswollen sperm heads in the egg cytoplasm and still more spermatozoa in the perivitelline space. Other systems in the egg cytoplasm were evidently undergoing maturation during the follicular phase, as judged by the failure of sperm heads within the vitellus of primary oocytes to decondense and form pronuclei. This metamorphosis is thought to be promoted by interaction between the sperm head and egg cytoplasm[12–14], and is an essential step in the formation of a spherical pronucleus.

Assay of samples of follicular fluid revealed a marked variation in the concentration of oestrogens between follicles from the same animal, ranging from 15–210 ng ml^{-1} on day 17 of the cycle to 108–750 ng ml^{-1} on day 20, shortly before the onset of behavioural oestrus. There was also considerable variation between animals in the oestrogen content of follicular fluid aspirated on the same day of the cycle. Nevertheless, an overall trend of an increasing concentration of oestrogens in follicular fluid could be plotted from day 17 (mean 75.3 ± 8.7 ng ml^{-1}) to day 20 (263.8 ± 54.5 ng ml^{-1}), these values decreasing only after the onset of oestrus or HCG injection. Twenty-four hours after the latter event(s), the concentration of follicular oestrogens was frequently reduced to 6–40 ng ml^{-1} (mean 20.7 ± 5.1 ng ml^{-1}), whereas progesterone titres were as high as 3,400–6,400 ng ml^{-1}. The corresponding values for the concentration of oestrogens in peripheral plasma increased from 42 pg ml^{-1} on day 17 to 190 pg ml^{-1} on day 20, decreasing to 25–30 pg ml^{-1} 24 h after the onset of oestrus.

These observations show that nuclear and cytoplasmic maturation of the oocyte are not essential concomitants of the final maturation of the Graafian follicle, at least when ovulation is induced by HCG in the mid-follicular phase of the oestrous cycle. The dissociation of the response of the somatic cells from that of the germ cell obtained in such a situation makes it reasonable to question any direct involvement of the oocyte in regulating the destiny of the follicle and its membrana granulosa[15], as suggested by studies of pigs and rabbits involving ovectomy[16,17]. and of rats involving cultured oocytes and granulosa cells[18].

The basic question arising from this work is the manner in which the oocyte normally responds to gonadotropic stimulation, and the nature of the failure to resume meiotic maturation in conditions of precocious ovulation induced by HCG. Previous work has demonstrated receptors for LH on porcine granulosa cells[19], and also that HCG can bind to these cells[16,20]. Accordingly, we propose that the increasing titres of intrafollicular oestrogens that arise during the latter part of the oestrous cycle mediate in the response of the oocyte to the gonadotropin stimulus, in part at least by increasing the binding sites available for LH or HCG on the granulosa cells; these sites have been reported to be similar[21]. Failure of the oocyte to respond after day 17 HCG injection is not due to a lack of gonadotropic hormone reaching the granulosa cells, since high levels of LH activity have been detected in follicular fluid within 4 h of injection of HCG (unpublished results of W. R. Carr and R.H.F.H.). In a broader biological context, the proposed involvement of oestrogens within the ripening follicle seems fundamental, since oocyte maturation would normally occur only in circumstances in which the animal was exhibiting behavioural oestrus, and where the reproductive tract was tonic and oedematous. In other words, an integrating gonadal hormone is needed during the late follicular phase to coordinate changes within the follicle, the reproductive tract and the hypothalamo-pituitary axis.

We thank the Wellcome Trust for support. We also thank Mr R. Nichol and Mrs Anne Kelly for technical assistance.

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Received December 12, 1975; accepted January 28, 1976

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Bilateral electroencephalographic response and unilateral tolerance to unilateral intracerebral morphine injections

DRUG tolerance is characterised by a loss of sensitivity to the pharmacological properties of a drug as a result of repeated exposure. Because it can be produced by both intravenous and intracerebral injection of opiates or barbiturates[1–3], functional alteration of neural cells probably contributes to its development. The identification of mechanisms underlying the development of tolerance to morphine in the central nervous system is complicated by secondary drug-induced changes in bioelectrical and metabolic activity. This can be circumvented by the use of a mirror-focus preparation where comparison of the drug-treated region in one hemisphere can be made with the morphological homologue in the contralateral hemisphere. For example, Morrell[4] induced bilateral epilepsy after unilateral treatment with an irritant that causes the epileptic discharge pattern to be propagated, through the commissural projections, to the mirror-focus cells in the opposite hemisphere. Since the mirror-focus cells are never in direct contact with the epileptogenic agents, any alteration in their metabolism is due solely to epilepsy. Similarly, seizures generated by electrical stimulation of one amygdala propagate to the contralateral amygdala[4,5]. Since the anterior amygdala of the rat has a high concentration of opiate receptors[6], we have used it to investigate the effects of direct unilateral injection of morphine sulphate. We found that injections of opiates such as morphine and levorphanol produce seizures at the injection site that propagate to the mirror-focus in the contralateral hemisphere. Tolerance to opiates develops at the injection site but the mirror-focus retains normal sensitivity to opiates in spite of the shared epileptiform activity.

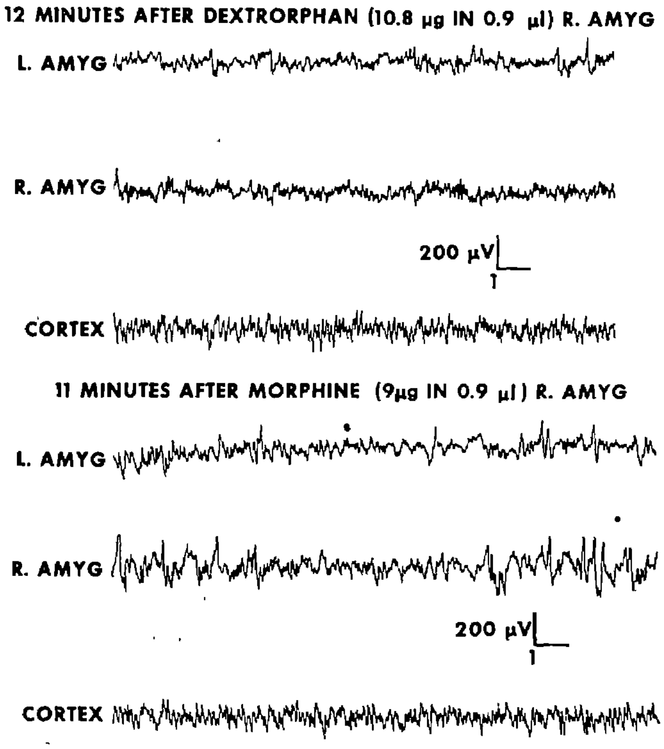

Fig. 2 Comparison of the effects of morphine and dextrorphan injected at the same site. Spike and wave pattern is evident after morphine. Dextrorphan has no effect. Cortical tracings are unaffected by amygdala injections.

Twenty-two Sprague–Dawley rats were anaesthetised with sodium pentobarbital and bilateral cannula-recording electrode assemblies were implanted in the anterior amygdala. The permanently implanted cannulae serve as a guide for smaller fluid delivery cannulae and as one pole of the bipolar recording assembly that monitors changes in electrical activity at the injection site. Injections of opiates

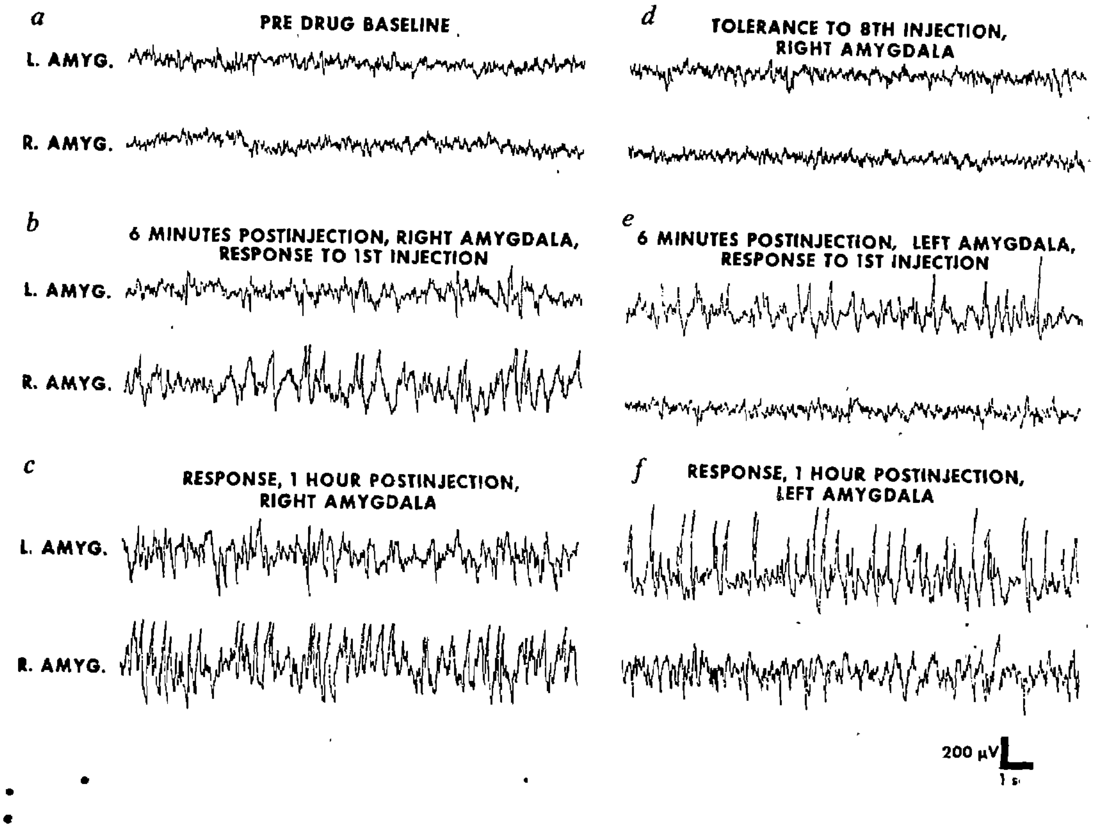

Fig. 1 Lateralisation of morphine tolerance to the right amygdala as a result of eight consecutive microinjections (2.7 μg in 0.9 μl) of morphine. In spite of the shared epileptiform activity of the mirror-focus in the left hemisphere, tolerance to morphine is restricted to the original injection site.

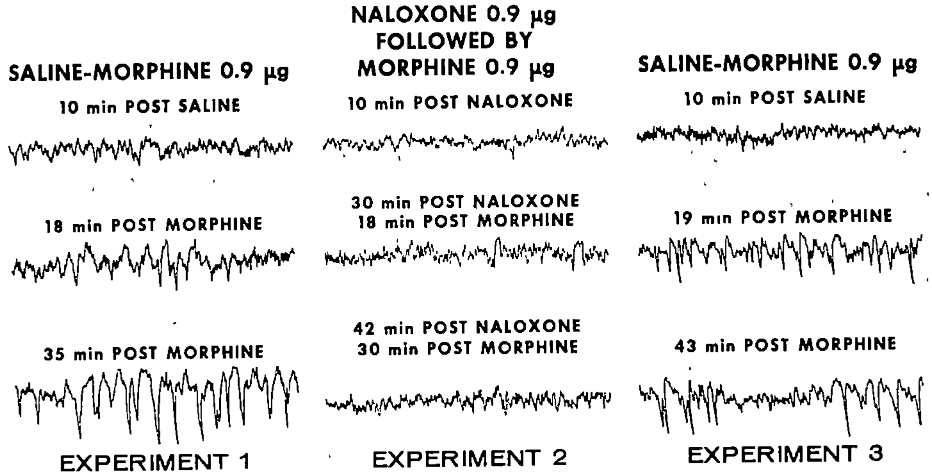

Fig. 3 Antagonism of morphine-induced changes in amygdala electroencephalographic activity by preinjection of an equal dose of naloxone. Experiments 1 and 3 were carried out 2 d before and after naloxone challenge.

and control injections of saline were delivered at a rate of 0.03 μl s^{-1} during 30 s.

After injecting morphine sulphate (2.7 or 9.0 μg) unilaterally into the anterior amygdala, we observed a spike and wave pattern at the injection site in 17 of 22 animals (Fig. 1*a* and *b*). The pattern appeared at the contralateral amygdala 15–30 min after injection and persisted for 3–4 h (Fig. 1*c*) each day. Injections of saline had no effect at either recording site. After repeated daily injections, tolerance manifested by a diminished bioelectric response developed at the injection site (Fig. 1*d*).

In spite of the daily appearance of sustained epileptiform patterns of the mirror-focus in the opposite hemisphere, tolerance was not transferred to the opposite hemisphere. When a similar dose of morphine was administered directly to the previously untreated amygdala, sensitivity to the drug was normal at the mirror-focus (Fig. 1*e*). Furthermore, kindled spike and wave patterns were seen at the original injection site (Fig. 1*f*). Levorphanol (3.0 μg) caused a similar response when injected into the amygdala. Furthermore, cross tolerance between the two opiates has been observed (our unpublished observations). Dextrorphan (10 8 μg), however, elicited no apparent electroencephalographic response (Fig. 2).

Several lines of evidence indicate that the electroencephalographic response of the amygdala elicited by acute morphine administration, and the eventual disappearance of this response with chronic morphine treatment is not a toxic or nonspecific effect

Cannula location is critical in the production of this response. Histological examination showed that cannulae placed bilaterally over the anterior amygdala gave bilateral opiate responses. If the tips of the cannulae were in the ventrolateral striatum or deep in the pyriform cortex, no morphine response was seen. The localisation of this response to the anterior amygdala is consistent with reports of high concentrations of opiate receptors in this region.

Although chronic morphine administration led to diminution of the response of the amygdala, an increase in the dose resulted in the reappearance of the response seen with acute doses. The loss of sensitivity seen after repeated injections of morphine could not have been the result of consequent tissue distortion, because a week of daily control injections of an equal volume of saline had no effect on morphine sensitivity of the amygdala ($N=3$). The response to the opiates is stereospecific—levorphanol but not dextrorphan mimics the action of morphine Naloxone has no apparent effect when administered by itself but blocks the effect of subsequent morphine administration. Pretreatment with an equal dose of naloxone (10 min before morphine) completely blocked the morphine response (Fig. 3) seen on control days before (experiment 1) and after (experiment 3) the naloxone test when sensitivity to morphine was normal ($N=4$).

Evoked potential thresholds (produced by electrical stimulation of the contralateral amygdala) were unchanged in spite of the appearance of drug-induced alterations in electroencephalogram patterns at the injection site. Recordings always returned to baseline after drug administration.

These findings show that unilateral tolerance can be produced by unilateral intracerebral microinjection of morphine sulphate into the amygdala Furthermore, the morphine-induced epileptiform patterns shared by both amygdala do not contribute to the development of drug tolerance

Although tolerance to direct intracerebral injections of opiates and barbiturates has already been demonstrated[1,3], this is the first demonstration of lateralisation of opiate tolerance. We plan to relate this unilateral alteration in drug sensitivity to an ipsilateral alteration in biochemical activity that is not seen at the contralateral site in the brain in spite of identical morphology and shared epileptiform experience.

We thank Endo Laboratories and Hoffman La Roche for naloxone, dextrorphan and levorphanol.

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Changes in sensitivity of morphine-induced circling behaviour after chronic treatment and persistence after withdrawal in rats

BEHAVIOURAL effects of morphine may be mediated, at least in part, by functional changes in the dopaminergic nigrostriatal system[1]. Thus, for example, acute administration of morphine increases the rate of dopamine turnover in the striatum (for examples refs 2–4), and nigrostriatal lesions block symptoms of morphine withdrawal[5–7]. Spontaneous turning in circles after drug treatment in rats has been reported to be an index of an intrinsic asymmetry between left and right nigrostriatal pathways[8–12]. We now report that both acute morphine administration and cessation of chronic morphine administration induce this rotation, which persists for at least 2 months after withdrawal. The data suggest that morphine induces the synthesis of an endogenous morphine-like substance[13–15] which persists long after morphine is eliminated.

The subjects were naive female Sprague–Dawley rats weighing approximately 260 g. For measuring rotation, rats were placed individually in a completely automated rotometer[16] which differentiates between complete 360° rotations and incomplete oscillatory turns. After habituation for 15 min each rat was injected intraperitoneally with morphine sulphate or saline. Rotations were recorded on a printout counter during the 60 min after injection. Rotations to the left or right were totalled separately and the net positive rotational difference (that is rotations in the dominant direction minus rotations in the opposite direction) was determined for each rat.

In the preliminary acute dose–response studies, doses from 0.25 to 20.0 mg kg^{-1} were tested (12 rats per dose). Morphine induced significant ($P < 0.05$, t tests) rotation at 2.5, 5 and 10 mg kg^{-1}; with larger doses rats became increasingly catatonic. Mean net rotations per hour ± s.e. for 0 (saline), 0.25, 1.0, 2.5, 5.0, 10.0 and 20.0 mg kg^{-1} were, respectively: 2.2±0.6, 5.8±1.4; 5.7±1.6, 7.2±2.3, 12.7±3.9, 10.0±2.6 and 4.8±1.4. As with other drugs[8,11], the rotation induced by morphine was consistent in direction and magnitude; that is, when retested at weekly intervals with 5.0 mg kg^{-1}, some rats consistently rotated to the right and others to the left, and the numbers of net rotations were significantly correlated ($r = +0.55$ to 0.61, $P < 0.01$, t test) from week to week. The rotation cannot be attributed to hyperactivity. If an animal were only hyperactive there might be more turns, but there is no reason *per se* why such turns should be unevenly distributed, in any consistent way, to the left or right. It seems that net rotations are inversely correlated with locomotor activity[17].

In the experiments involving chronic administration, 16 rats were initially (day 1) tested for rotation following an injection of morphine (5.0 mg kg^{-1}). A day later, silicone reservoirs were implanted subcutaneously in all rats as described by Goode[18]. On the next day, chronic morphine administration was begun for 3 d (days 3, 4 and 5). Each morning the reservoirs were rinsed with physiological saline and refilled with 0.5 ml of morphine sulphate solution in a saline vehicle (50 mg ml^{-1}). On the withdrawal day (day 6), the reservoirs were rinsed and filled only with saline. Immediately after removal of morphine from the reservoirs, each rat was placed in a rotometer and, 15 min later, injected with either morphine (5.0 mg kg^{-1}; group 1, $N = 8$) or saline (group 2, $N = 8$). Each rat was tested again for rotation after receiving the same dose of morphine or saline 1, 3, 7, 15, 30 and 60 d after withdrawal of chronic morphine (days 7, 9, 13, 21, 36 and 66 in Table 1). The body weight of each rat was recorded before the first injection of morphine (day 1) and on every day thereafter that rats were handled.

The results are shown in Table 1. After withdrawal of morphine on day 6, the rats in group 1 were somewhat tolerant to the rotatory effect of morphine and the rats in group 2 did not rotate after receiving saline. Mean net rotations in group 2 were not significantly different ($P > 0.1$, t test) from an acute effect of saline in naive rats ($N = 12$) nor from an effect of saline in rats implanted with reservoirs filled only with saline ($N = 4$). By 3 d after withdrawal (day 9), the rats injected with morphine were obviously more sensitive to the rotatory effect of morphine and the rats injected with saline rotated as much as they had previously in response to their first (day 1) injections of morphine. Both of these latter effects were greater 7 d after withdrawal (day 16) and remained stable thereafter on all subsequent testing days. In contrast, body weight changes showed a typical early withdrawal pattern[7,19]—a decrease in body weight 1–3 d after withdrawal followed by a return to pre-withdrawal weights within 7–10 d. The small numbers of net rotations (approximately two per hour) induced by saline in naive rats were not consistent in direction and did not increase in magnitude when rats were retested at weekly intervals for up to 2 months. Since saline does not normally induce rotation, withdrawal-induced rotation seems to be a genuine manifestation of a protracted abstinence syndrome[20].

Correlations of day 1 morphine-induced rotation with withdrawal-induced rotation, as well as with morphine-induced rotation after withdrawal, suggest that chronic administration of morphine induces the synthesis of a morphine-like substance[13–15] responsible for the prolonged rotatory effects observed. That is, the direction of rotation of each rat was consistent across all tests, and saline and morphine data from 3 to 60 d after withdrawal were quantitatively correlated ($r = +0.64$ to 0.71, $P < 0.01$, t test) with day 1 data; the more a rat rotated in response to an acute injection of morphine, the more it also rotated during the withdrawal tests. In previous studies[21] with other drugs, we have generally found that although rotation is always consistent from week to week, when rats are retested with the same drug, there are no positive and quantitative correlations between drugs (for example, rats may rotate in opposite directions with (+)-amphetamine and apomorphine)—such differences probably reflect different ways in which drugs can effect the intrinsic cerebral asymmetry[21]. The correlation between acute morphine-induced rotation and withdrawal-induced rotation suggests, therefore, that long after morphine is eliminated an endogeneous substance persists which has at least one pharmacological action resem-

Table 1 Rotation and body weight after chronic morphine administration

	Group 1			Group 2		
Day	Treatment	Rotation	Body weight	Treatment	Rotation	Body weight
1	M*	100†	100‡	M	100†	100‡
2	Surgery	—	—	Surgery	—	—
3–5	Chronic M	—	—	Chronic M	—	—
6	Withdraw M;M	71.9±22.2	105.5±1.5	Withdraw M;S	31.9±15.3	104.7±1.3
7	—	—	—	S	66.7±12.5	99.6±1.1
9	M	180.7±35.4	97.8±2.0	S	101.4±29.2§	99.6±1.3
13	M	249.0±42.6	101.4±1.9	S	220.8±36.5	106.7±1.0¶
21	M	141.7±41.6	106.4±2.1¶	S	158.3±47.4	112.8±1.5
36	M	231.8±37.1	116.8±2.0	S	240.3±41.6	122.3±2.0
66	M	172.2±33.8	131.3±2.1	S	170.8±31.7	131.8±2.1

Rotation and body weight are expressed as mean percentage of day 1±s.e.

*M alone refers to morphine sulphate, 5.0 mg kg^{+1}, intraperitoneal; chronic M or withdraw M refers to morphine administered through implanted (day 2) silicone reservoirs; S refers to saline, 0.1 ml, intraperitoneal.

†Mean net rotations per hour ± s.e. for all rats on day 1 were 11.2±4.8.

‡Mean body weight (g)±s.e for all rats on day 1 was 255.6±3.9.

§For all data points except this one, rotation was significantly different ($P < 0.05$–01, t tests) from day 1.

¶For all data points except these, body weight was significantly different ($P < 0.05$–01, t tests) from day 6 (pre-withdrawal).

bling that of morphine. This interpretation is consistent with the evidence that rotation is indicative of dopaminergic striatal function[8–12], that morphine is known to affect dopaminergic mechanisms in the striatum[1] and that the highest concentrations of opiate receptors[22] and endogenous morphine-like substances[13–15] are found in the striatum. Although there are other reports[23–25] of long term after effects of chronic morphine administration, the study reported here provides the first demonstration of an after effect resembling an acute effect of morphine.

This research was supported by a grant and a research scientist development award from the National Institute on Drug Abuse.

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Received November 7, 1975; accepted January 19, 1976

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Lithium differentially antagonises self-stimulation facilitated by morphine and (+)-amphetamine

IN spite of widespread use of lithium (Li) in psychiatry, the mechanism underlying its antimanic action is poorly understood. Studies of the effects of Li on spontaneous and drug-induced behavioural activity have yielded conflicting results[1–8]. Electrical self-stimulation of the brain (SS) may be a promising alternative approach to such studies This behaviour is believed to be mediated by catecholamines (reviewed in ref. 9), which have also been implicated in the action of Li[10–14]. Investigations of the effects of Li on SS have been restricted to a small range of doses and to SS in lateral hypothalamus, and the results have not been consistent[15,16]. We have now used a wider range of doses to investigate the effects of acute and chronic Li on SS in the substantia nigra of rats

Electrodes were implanted stereotaxically in substantia nigra (2.2 mm anterior to lambda, 1.5 mm lateral to midline, 8 mm ventral to the skull surface) of 100–120-d-old male albino rats (Carworth Farms) as described before[17] One week after surgery, SS tests began in operant conditioning chambers which have been described before[17]. The brain was stimulated by sine wave a.c. current (0.2 s duration). Rats were required to exceed a criterion performance of 1,100 bar presses per 30 min when reinforced by optimal current intensities (typically 40–60 μA r.m.s.). Most rats performed at considerably higher rates. All screening and drug SS sessions lasted 30 min. After completion of drug treatments, rats were killed and electrode placements were verified as before[17] (Fig. 1)

Before treatment with Li, current intensity was adjusted to yield an average SS rate of between 1,200 and 1,800 bar presses per 30 min. After three consecutive test sessions in which responding remained within these limits, bacteriostatic saline or Li (1.5, 2.0 or 2.5 meq kg^{-1}) was administered as described in Fig. 2. Lithium chloride (Matheson, Coleman and Bell) was discovered in sterile distilled water and injected subcutaneously in a volume containing 2 ml kg^{-1}. The solution was halved and injected into different sites. Skin ulceration was never observed as a result of injection of Li.

The effects of Li on rats self-stimulating at a lower rate were also investigated. After current intensity had been reduced to yield baseline SS rates between 450 and 950 bar presses per session, Li (2.0 meq kg^{-1}) was administered and testing was conducted as in Fig. 2.

Neither saline nor Li at 1.5 meq kg^{-1} significantly changed SS from baseline responding rates (Fig 2). The small reduction of SS induced by Li at 2.0 meq kg^{-1}, was statistically significant on days 3 and 5 of treatment, but not on days 7 and 10, suggesting the development of partial tolerance to the initial depressant effect of Li on SS. Although Li at 2.5 meq kg^{-1} significantly reduced SS on days 3–10 of treatment, the large progressive reduction in SS was accompanied by an average weight loss of 9% in 10 d of treatment ($t=7.73$, $P<0.01$ for the comparison between body weight before Li and on day 10 of treatment). Concomitant lethargy was seen in some animals and it seemed possible that the effects of Li at 2.5 meq kg^{-1} on SS might have been confounded with nonspecific toxic effects of this dose. In contrast, Li at 2.0 meq kg^{-1} did not reduce body weight significantly. Gross observations failed to distinguish animals treated with Li at 2.0 meq kg^{-1} from controls except for polyuria in some animals. as described by Schou[18]. The animals self-stimulating at a lower baseline rate (Fig. 2) also reduced SS on day 5 of treatment and subsequent recovery was apparent. Thus, the effects of Li on SS did not seem to be rate specific. These results are compatible with the report that Li increased electrical current thresholds for SS 24 h after injection[16]

To evaluate the mechanism by which Li alters SS, its interactions with morphine and (+)-amphetamine were studied Both morphine and (+)-amphetamine facilitate SS[20–22] Although the mechanism by which these drugs enhance SS has yet to be fully elucidated. there is some evidence that their effects on behaviour involve different neuropharmacological mechanisms[23,24]

Before initiation of chronic treatment with morphine. current intensity was reduced to yield SS between 450 and 950 bar presses per 30 min. After three consecutive test sessions of responding within these limits, morphine (Mallinckrodt. 15 mg kg^{-1}, subcutaneously in saline) was administered daily After 9 d 19 of 24 rats had each increased SS to at least 50% above baseline responding These 19 rats were then matched for response rates on day 9 of chronic treatment with morphine and were injected subcutaneously with saline, Li at 1.5 meq kg^{-1}, or Li at 2.0 meq kg^{-1} The remaining five rats reduced SS during chronic treatment with morphine and were eliminated from the study.

Li significantly reduced morphine-facilitated SS throughout treatment (Fig. 3) At no time during combined Li (2.0 meq kg^{-1}) and morphine treatment did any animal regain the SS rate which had been attained on day 9 of treatment with morphine alone. After cessation of treatment with Li (2.0 meq kg^{-1}) on day 22, SS rates increased in five of six rats and on day 25 of continued treatment

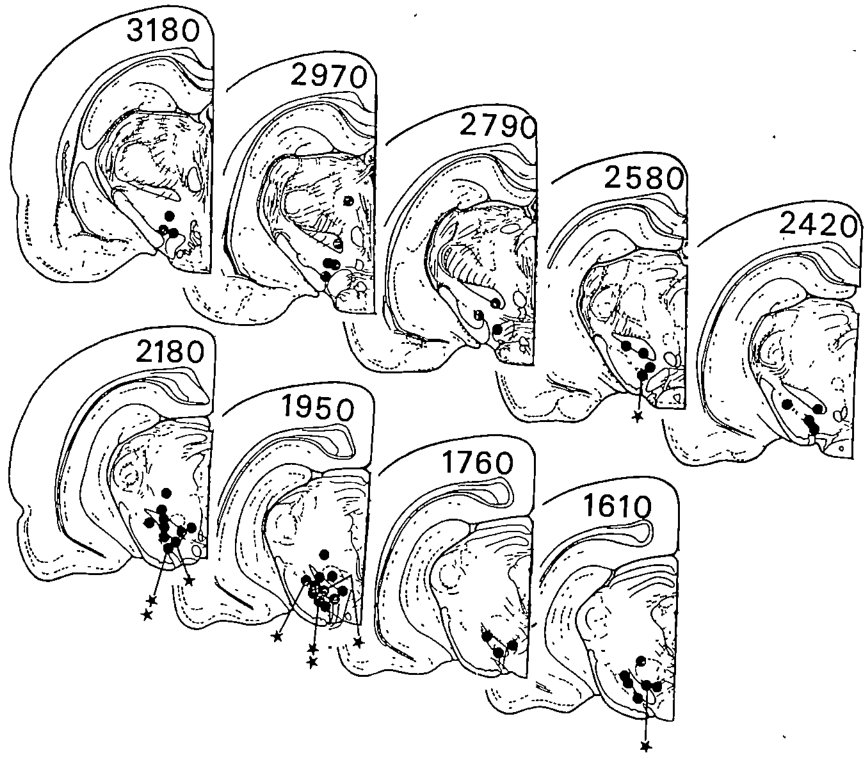
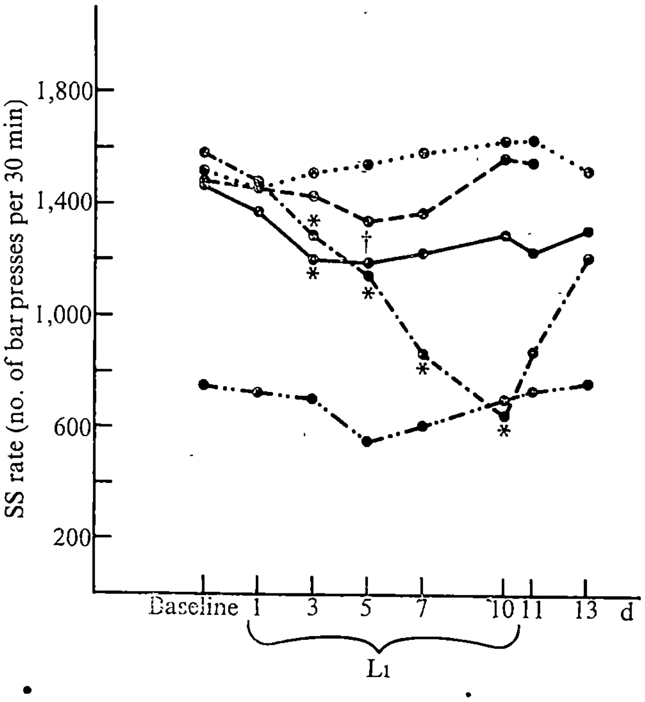


Fig. 1 Electrode placements of self-stimulating animals. Frontal sections are modified from Konig and Klippel[18]. Because of causes unrelated to the nature of the experimental treatment, histological results were unavailable for three rats. Some placements were slightly anterior to substantia nigra and others fell slightly dorsal to this structure, but there was no apparent relationship between electrode placement and experimental results. ★, Two overlapping electrode tip placements; ★★, three overlapping placements.

with morphine, SS did not differ significantly from those after morphine alone on day 9. Neither saline nor Li at 1.5 meq kg^{-1} significantly altered morphine-facilitated SS on any day of testing.

The observed antagonism of morphine-facilitated SS by Li agrees with the report[25] that Li reduces oral morphine consumption in morphine-dependent rats and that tolerance does not seem to develop to this effect. One possible explanation for the effects of Li on morphine-facilitated SS is that Li interferes selectively with the penetration or distribution of the narcotic. This does not seem to be the case, however, because Li has been reported to enhance morphine-induced analgesia[26].

Fig. 2 Effects of chronic Li treatment on SS. Test sessions for SS took place 2 h after injection of Li on days 1, 3, 5, 7, and 10 of treatment. The rats were retested for SS on the first and third days after cessation of Li treatment (days 11 and 13). · · · · ·, Saline (n = 8); – – –, Li, 1.5 meq kg^{-1} (n = 8); ——, Li, 2.0 meq kg^{-1} (n = 8); – · – ·, Li, 2.5 meq kg^{-1} (n = 6); – · –, Li, 2.0 meq kg^{-1}, low rate (n = 3) $*P < 0.05$ for comparison between the indicated data point and preceding baseline. $\dagger P < 0.01$.



Before treatment with (+)-amphetamine, current intensity was reduced in the same manner as for combined treatment with morphine and Li. After responding stabilised, (+)-amphetamine (Sigma, 0.5 mg kg^{-1}, free base) was administered subcutaneously 30 min before testing (days 1–17, Fig. 4). Li was administered daily from days 5 to 14 (Fig. 4). Chronic Li (2.0 meq kg^{-1}) did not alter SS significantly on any day of treatment with Li and (+)-amphetamine (Fig 4). Because the effects of (+)-amphetamine on SS in substantia nigra are not altered by repeated administration[17], it was not necessary to control for this possibility.

The lack of antagonism of (+)-amphetamine-facilitated SS by Li contrasts with the reported reduction of (+)-amphetamine-induced hyperactivity by Li[8] These results further support the suggestion that different neurochemical mechanisms mediate these behavioural activities[22].

Because both the nigro-neostriatal dopamine pathway[22,27,28] and the dorsal and ventral noradrenaline pathways[29,30] have been proposed as substrates for SS in substantia nigra, it is not possible to state which of these pathways are involved in the effects reported here. Regardless of the pathway involved, these results suggest that (+)-amphetamine- and morphine-induced facilitation of SS are mediated by different mechanisms of catecholamine neurotransmission. Enhancement of activity and SS by low doses of (+)-amphetamine is believed to result from facilitation of catecholamine release[20,24,31] from a newly synthesised pool[32,33]. In contrast, the effects of morphine on activity seem to depend on a reserpine-sensitive pool of stored catecholamines[34,36]. If, therefore, Li interferes with catecholamine storage as suggested[12], morphine- but not (+)-amphetamine-facilitated SS would be expected to be reduced by Li.

Regardless of the neurochemical mechanisms involved, the differential effects of Li on morphine- and (+)-amphetamine-induced facilitation of SS may have important clinical implications If the drug abuse potential of morphine and amphetamine is related to the activation of brain reward pathways, then the results obtained with

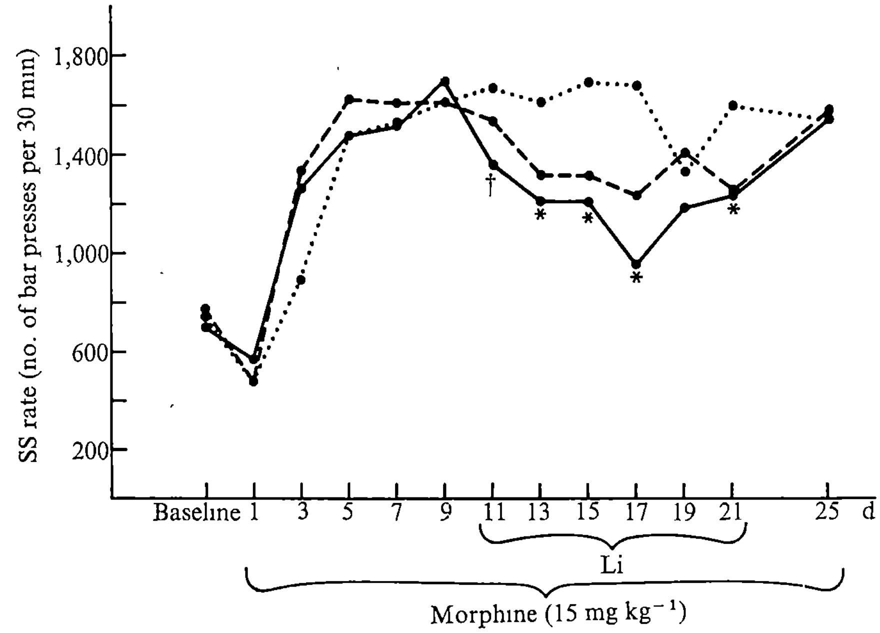


Fig. 3 Effects of chronic Li treatment on morphine-facilitated SS. Morphine was injected daily (days 1–25). Test sessions for SS took place on alternate days 3 h after injection of morphine. From days 11 to 21 Li was administered daily 1 h after morphine. The effects of Li on SS facilitated by morphine were assessed by comparison with responding on day 9. · · · · · , Saline+morphine ($n = 7$); – – –, Li, 1.5 meq kg^{-1}+morphine ($n = 6$); ——, Li, 2.0 meq kg^{-1}+morphine ($n = 6$). *$P < 0.05$ for the difference between the data point and the corresponding score on day 9. †$P < 0.01$.

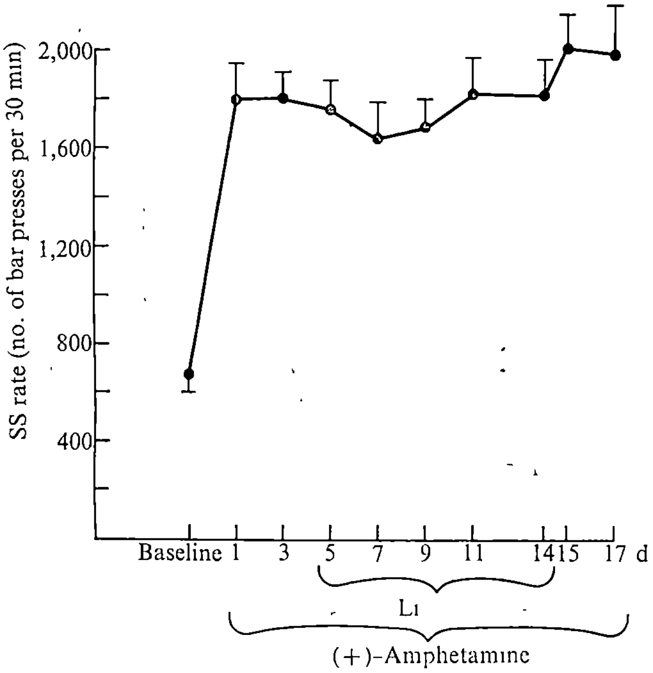


Fig. 4 Effects of chronic Li treatment on (+)-amphetamine-facilitated SS ($n = 8$). (+)-Amphetamine was administered 30 min before each test session on days 1–17. Li (2.0 meq kg^{-1}) was administered daily 2 h before testing on days 5–14. Self-stimulation during combined Li and (+)-amphetamine administration was compared with responding on day 3.

SS may have practical applications. Thus, for example, on the basis of our results it may be suggested that Li will be effective in the treatment of morphine, but not amphetamine, addiction.

We thank Peter Binstock and David Benkle for technical assistance. This research was supported by an NIH research scientist award (to D.S.S.), an NIH post-doctoral research fellowship (to J.M.L.) and a grant from the US Public Health Service.

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Received September 29, 1975; accepted January 14, 1976.

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Histamine-sensitive adenylate cyclase in mammalian brain

THERE is increasing interest in the possibility that histamine may be a neurotransmitter in the mammalian central nervous system (CNS). Electrophysiological evidence supports the existence of histamine receptors in nervous tissue through which neuronal excitability can be regulated. For example, the firing rate of certain neurones in the brain is depressed by histamine and this depression is specifically blocked by the H_2 antagonist, metiamide[1]. In addition, histamine influences the electrical excitability of neurones in the superior cervical ganglion[2]. Certain neurotransmitters may exert some of their effects in nervous tissue by stimulating the formation of cyclic AMP. It is therefore of interest that histamine raises cyclic AMP levels in brain slices[3–7], as do several other putative neurotransmitters, such as noradrenaline, dopamine and 5-hydroxytryptamine (5-HT). Furthermore, a dopamine-sensitive adenylate cyclase

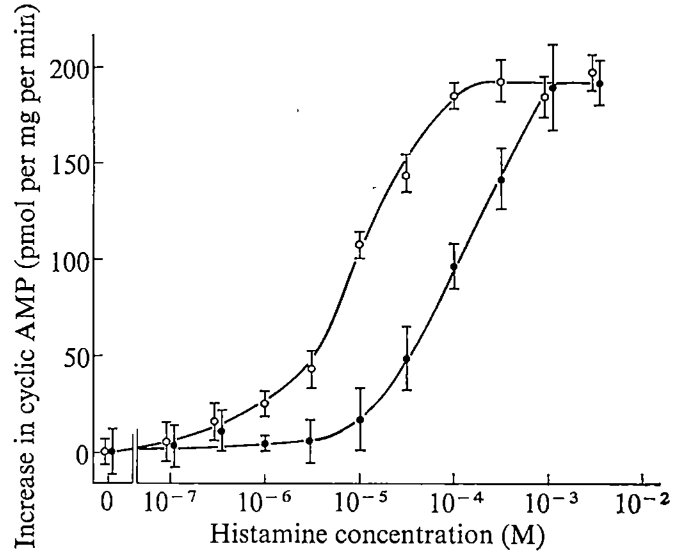

Fig. 1 Effect of various concentrations of histamine in the absence (○) or presence (●) of 10^{-5} M metiamide, on adenylate cyclase activity in homogenates of dorsal hippocampus from guinea pig brain. Adult female Hartley guinea pigs were killed by decapitation. The dorsal hippocampi were dissected, weighed, and manually homogenised in 150 volumes ice-cold 2 mM Tris maleate–2 mM EGTA, *p*H 7.8, using a glass homogeniser with a Teflon pestle. The adenylate cyclase reaction mixture contained the following final concentrations (mM): Tris maleate (*p*H 7.8), 100; EGTA, 0.6; 3-isobutyl-l-methylxanthine, 1; $MgCl_2$, 2; ATP, 1; GTP, 0.1; plus test substances as indicated, in a final volume of 500 μl. The enzyme reaction, initiated by the addition of 50 μl homogenate, was carried out for 6 min at 30 °C, and was terminated by placing the assay tubes in a boiling water bath for 2 min. Aliquots (50 μl) were assayed in duplicate for cyclic AMP by the method of Brown *et al.*[12]. Protein was determined by the method of Lowry *et al.*[13]. In the absence of added histamine or metiamide, 153±4 pmol cyclic AMP per min per mg protein were formed; in the presence of 10^{-5} M metiamide, 152±10 pmol cyclic AMP per min per mg protein were formed. The increase in cyclic AMP above basal level is plotted as a function of histamine concentration. Values represent the mean ± s.e.m. of nine replicate samples in the absence, and three replicate samples in the presence, of histamine.

has been found in homogenates of rat caudate nucleus and olfactory tubercle, with properties very similar to those of the dopamine receptor in mammalian brain[8–10]. We report here the occurrence of an adenylate cyclase which is activated by low concentrations of histamine in several regions of guinea pig brain. This histamine-sensitive adenylate cyclase has the pharmacological properties of an H_2 receptor. The results indicate that the actions of histamine on H_2 receptors in the CNS may be mediated through cyclic AMP.

In the present study, 100 μM histamine caused maximal stimulation of adenylate cyclase in homogenates of guinea pig dorsal hippocampus. In several experiments, the maximal increase in adenylate cyclase activity varied from 70–200%. Addition of GTP to the reaction mixture increased enzyme activity and markedly potentiated the stimulation of adenylate cyclase by histamine. There results are consistent with other studies indicating that GTP and its analogues can regulate the activity of adenylate cyclases[11].

Figure 1 illustrates the effects of various concentrations of histamine on adenylate cyclase activity in homogenates of dorsal hippocampus from guinea pig brain. The concentration of histamine required for half-maximal activation (K_a) of the enzyme was 8 μM. This agrees well with the K_a values of 7 and 9 μM reported, respectively, for histamine-induced cyclic AMP formation in brain slices[6] and in cultures of intact human astrocytoma cells[14].

Two types of histamine receptors have been characterised in mammalian tissues[15,16]. H_1 receptors are selectively blocked by classical anti-histaminic agents such as mepyramine and promethazine, whereas H_2 receptors are selectively blocked by recently developed H_2 antagonists such as metiamide, cimetidine and burimamide[17]. As shown in Fig. 1, metiamide acted as a competitive inhibitor of the activation by histamine of adenylate cyclase. In the presence of 10 μM metiamide, the apparent K_a for histamine was increased to 100 μM. From these data, the K_i value[18] of the enzyme for metiamide was calculated to be 0.87 μM, which is in excellent agreement with the K_i values for metiamide inhibition of H_2 receptors calculated from measurements of physiological responses using guinea pig atrium (0.92 μM) and rat uterus (0.75 μM)[17]. In contrast, the H_1 antagonist mepyramine, at 1 μM (a concentration which maximally inhibits the effects of 100 μM histamine on H_1 receptors[6]), had no effect either on the K_a for histamine or on the maximal stimulation of adenylate cyclase. Other agents which antagonise the actions of various neurotransmitters on their receptors were tested for possible effects on adenylate cyclase activity in the absence and presence of 100 μM histamine—propranolol, phentolamine, atropine, and fluphenazine, in concentrations of 10 μM, had no effect on basal or histamine-stimulated enzyme activity.

The ability of two substances known to act as agonists at histamine receptors were compared with histamine as activators of adenylate cyclase (Fig. 2). 4-Methyl-histamine increased adenylate cyclase activity with half-maximal stimulation occurring at 23 μM. This compound has been reported to have 25–43% of the potency of histamine in activating H_2 receptors, but negligible activity at H_1 receptors[16]. In contrast, 2-methyl-histamine primarily affects H_1 receptors. It weakly activates H_2 receptors as well, having 2–4% of the potency of histamine in this regard. In agreement with these properties, 2-methyl-histamine stimulated adenylate cyclase, but only at high concentrations (K_a=120 μM). Thus, the pharmacological properties of the histamine-sensitive adenylate cyclase seem to be those of an H_2 receptor.

The regional distribution of histamine-sensitive adenylate cyclase activity in rat and guinea pig brain is shown in Table 1. In guinea pig brain, histamine markedly stimulated adenylate cyclase in homogenates from hippocampus, neocortex, and corpus striatum, but did not significantly affect enzyme activity in other brain regions. This localisation of histamine-sensitive adenylate cyclase activity correlates well with the regional distribution of histamine-induced cyclic AMP accumulation in brain slices[7]. Of the various regions of rat brain examined, histamine-stimulated adenylate cyclase only in neocortex, where a small but statistically significant increase in activity was seen. An histaminergic pathway which projects to the cortex in rat brain has recently been described[19]. The histamine-sensitive adenylate cyclase present in rat neocortex may represent the postsynaptic receptor of neurones in this region receiving

Fig. 2 Effect of various concentrations of histamine (○), 4-methyl-histamine (△), and 2-methyl-histamine (×) on adenylate cyclase activity in homogenates of guinea pig dorsal hippocampus. Experimental procedures were as in Fig. 1. The maximal stimulation of adenylate cyclase activity by saturating concentrations of histamine, 4-methyl-histamine, and 2-methyl-histamine was the same within the limits of experimental error.

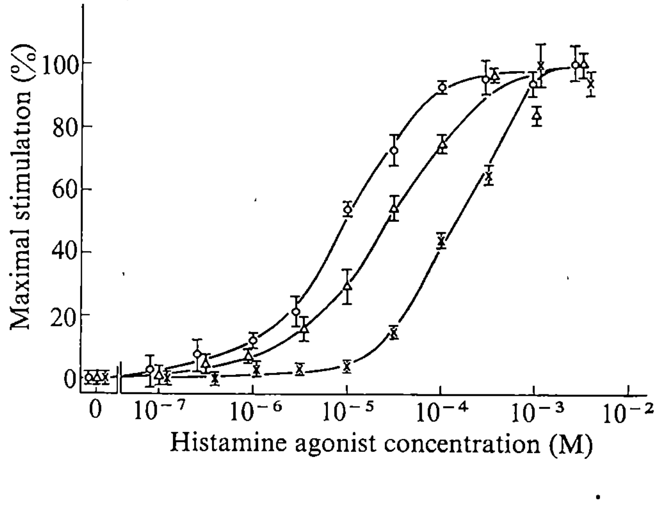

Table 1 Regional distribution of histamine-sensitive adenylate cyclase activity in guinea pig and rat brain

Brain region	Adenylate cyclase activity* (pmol per min per mg protein) −Histamine	+Histamine (100 μM)
Guinea pig		
Dorsal hippocampus	106±4	218±8†
Ventral hippocampus	111±6	199±6†
Neocortex	98±4	187±7†
Corpus striatum	101±5	165±3†
Cerebellum	33±3	35±2
Hypothalamus	157±4	158±6
Thalamus	65±5	71±3
Midbrain	127±4	129±3
Pons	159±8	156±5
Medulla	118±6	125±4
Optic chiasm	46±9	44±6
Rat		
Hippocampus	63±3	67±6
Neocortex	54±2	65±3†
Corpus striatum	72±6	74±3
Cerebellum	73±5	78±6

*Experimental procedures were as described in Fig. 1. Values represent mean ± s.e.m. of four replicate samples.

†Activity in the presence of histamine was significantly different ($P < 0.005$) from that in the absence of histamine.

histaminergic innervation. The localisation of histaminergic pathways in guinea pig brain has not yet been determined.

The non-uniform distribution of histamine-sensitive adenylate cyclase activity in brain is of particular interest, since histamine has been shown to increase cyclic AMP in cultured cells derived from glial elements[14] as well as to increase adenylate cyclase activity in brain capillaries[20]. If the enzyme we are studying were primarily glial or vascular in origin, one would expect a fairly uniform distribution of histamine-sensitive adenylate cyclase activity throughout the brain. The non-uniform distribution of activity actually observed (Table 1) is more consistent with the possibility that the enzyme is located primarily in selected neurones which receive histaminergic innervation.

In studies of guinea pig neocortical and hippocampal brain slices, activation of either H_1 or H_2 receptors led to the accumulation of cyclic AMP[6,7]. The present results indicate that the H_2 receptor in brain, like the dopamine receptor[8–10], is intimately associated with an adenylate cyclase. The idea of a direct connection between H_2 receptors and adenylate cyclase in nervous tissue is supported by studies of heart[21,22] and stomach[23,24], in which histamine, acting on H_2 receptors, caused an increase in formation of cyclic AMP in both intact preparations and homogenates.

We have been unable, using homogenates of guinea pig hippocampus, to identify a histamine-sensitive adenylate cyclase which possesses the pharmacological properties of an H_1 receptor. It is possible that such an adenylate cyclase does exist in brain, but that it was either labile or inactive in the experimental conditions used. Alternatively, histamine, acting on H_1 receptors, may cause cyclic AMP accumulation in brain slices by an indirect mechanism. For example, histamine might cause the release of other neurotransmitters which, acting on their own target cells, could then activate adenylate cyclases. Clearly, the relationship of H_1 receptors to cyclic AMP metabolism requires further investigation. Interestingly, in non-neuronal tissues, an increase in cyclic GMP in response to activation of H_1 receptors has been reported[25,26].

The present results indicate that the H_2 receptor in mammalian brain may be the histamine-binding portion of a histamine-sensitive adenylate cyclase. One corollary of this conclusion is that the physiological consequences of histamine acting at H_2 receptors in the brain, such as depression of the firing rate of certain neurones[1], may be mediated through cyclic AMP. Further studies on the biochemistry and pharmacology of this histamine-sensitive adenylate cyclase, and its relationship to physiological processes in neurones, may help to clarify the role of histamine in the mammalian nervous system.

We thank J. Paul of Smith, Kline and French for histamine agonists and antagonists, and F. Wilson for assistance.

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Received December 9, 1975; accepted January 6, 1976.

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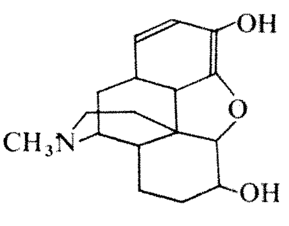
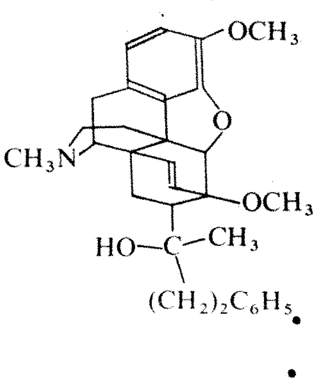
Biosynthetic origin and receptor conformation of methionine enkephalin

RECENT reports have shown that the brain contains an endogenous peptide with opiate-like activity[1–3] and similar peptides have been found in the pituitary[4,5,13]. One of the brain peptides, known as methionine enkephalin, was identified as a pentapeptide Tyr–Gly–Gly–Phe–Met[6], and evidence was presented that a minor component may have leucine in place of methionine. The principal sequence is identical to that at the NH_2-terminus of lipotropin C fragment, a peptide discovered in substantial quantity in porcine pituitary[7,8]. This suggests that methionine enkephalin is derived *in vio* by proteolytic cleavage of C fragment. Since enkephalin is thought to compete directly with opiates for the brain opiate receptor, while having no primary structure similarity to opiates, we have searched for and found a basis for the competition in a proposed secondary structure for the peptide.

Spatial models of morphine (I), oripavine (II), a potent derivative of morphine, and methionine enkephalin were





constructed. The conformation of morphine is essentially defined by the rigidity of its molecular structure and similar considerations apply to the assignment of conformation in oripavine. The pentapeptide, on the other hand, should be able to assume various conformations in aqueous solution but at the receptor a unique conformation would be expected. With methionine enkephalin a model could be constructed which presents structural elements in common with morphine; the proposed structure bears a striking resemblance to the spatial structure of oripavine (Fig. 1).

In the models, specific functional groups are held in the same orientation whether suspended from a peptide backbone or forming part of a rigid alkaloid structure. The tyrosine side chain and the amino group in enkephalin correspond exactly with the orientations of the phenolic ring in the phenanthrene nucleus and the tertiary amino group of oripavine. The two glycine residues occupy positions 2 and 3 in a β bend with a hydrogen bond between the carbonyl group of the tyrosine residue and the amino group of the phenylalanine, allowing the phenylalanine side chain to coincide with the positioning of the phenylethyl substituent at position 19 in the alkaloid. This lipophilic group, which is absent in morphine, could contribute to the stronger receptor binding of oripavine and enkephalin. The carboxyl terminal methionine residue lies between the aromatic side chains of tyrosine and phenylalanine; the methyl thioether chain then has the same orientation as the methyl ether substituent at position 6 in oripavine.

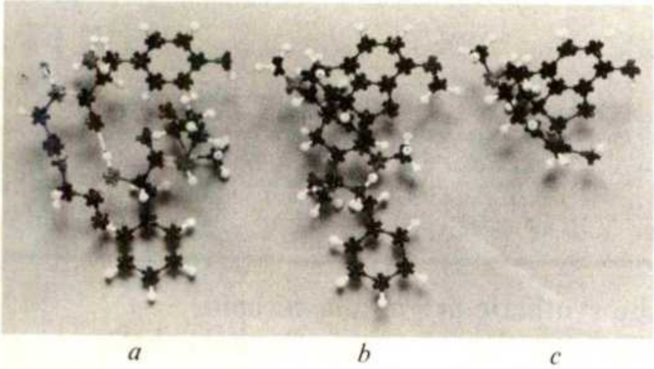


Fig. 1 Framework molecular models of tyrosylglycylglycylphenylalanylmethionine (methionine enkephalin) (*a*); oripavine (*b*), and morphine (*c*). The models were constructed from components described by Doré[12].

Evidence for the proposed conformation of the neuroactive pentapeptide emerged on application of empirical rules for predicting secondary structure from a knowledge of amino acid sequence. Both the Chou and Fasman rules[9,10] and the principles of Lewis, Momany and Scheraga[11] indicated a strong tendency for the NH$_2$-terminal region of the C fragment of lipotropin to form a β bend comprising the residues Tyr–Gly–Gly–Phe and this β bend is one of the structural elements in a model of lipotropin constructed in our laboratory (unpublished results). It is notable that the conformation proposed for the biologically active form of methionine enkephalin centres on a β bend involving the same four residues.

The molecular structures proposed for the opiate reactive peptide, morphine and oripavine provide an explanation for the fact that a characteristic biological activity is expressed by molecules entirely different in class and origin.

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Received December 27, 1975; accepted February 6, 1976.

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Gibberellic acid enhances the level of translatable mRNA for α-amylase in barley aleurone layers

α-AMYLASE is synthesised *de novo* in response to the plant hormone gibberellic acid (GA$_3$) in the aleurone layers of barley grains[1]. This enzyme induction depends on RNA and protein synthesis, as determined by the use of inhibitors[2–4], and it occurs after a temperature-dependent lag period[5]. The biochemical events of the lag period have been studied with respect to ionic requirements[5–7], early protein synthesis[8], RNA synthesis[9–12], polysome formation[13] and lipid synthesis and turnover[14–17]. It has been postulated that new mRNA for α-amylase synthesis might be produced in response to the hormone[2,3] and there is evidence that GA$_3$ enhances the incorporation of labelled ribonucleosides into poly(A)-containing RNA of barley aleurone layers[18–20]. Stimulation could be detected about 4 h after hormone treatment and although GA$_3$ probably stimulates synthesis of a limited number of proteins, there was stimulation of a wide size range of poly(A)-containing RNAs. Thus it was not possible to say that GA$_3$ had a selective effect on the synthesis of the mRNA for α-amylase.

In an attempt to relate GA$_3$-stimulated α-amylase production to the level of α-amylase mRNA, we have assayed for this mRNA by translating total and poly(A)-containing RNA isolated from aleurone layers in a cell-free system derived from wheat embryos[21]. This was followed by immunoprecipitation[22] and sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis[23] of the cell-free translation products. We found that the level of translatable mRNA for α-amylase increases in hormone-treated tissue in parallel with the increased rate of enzyme synthesis.

The template activity of total RNA from both control and hormone-treated tissue was low (fivefold stimulation) compared with tobacco mosaic virus (TMV) RNA[24] (50-fold stimulation). The aleurone RNA preparations, however, did not contain inhibitors of translation when assayed with TMV RNA-directed cell-free protein synthesis (unpublished). Total RNA isolated from aleurone layers incubated in the absence and presence of GA$_3$ for 8 h was used to program the wheat embryo cell-free system. The translation products were analysed by SDS–polyacrylamide gel electrophoresis followed by fluorography[26] of the dried gels and they varied in size from 8,000 to 70,000 daltons (Fig. 1*a* and *b*). In spite of the variety of proteins induced in aleurone cells by GA$_3$, there were relatively few detectable differences in the patterns of cell-free translation products when the system was programmed by RNA from GA$_3$-treated and untreated tissue. But cell-free synthesis of at least two polypeptides was increased by RNA from GA$_3$-treated tissue, one migrating as a broad band at about 35,000 daltons and one at 45,000 daltons. The former was not identified and may consist of several polypeptides. The 45,000-dalton polypeptide coelectrophoresed with α-amylase.

Poly(A)-RNA was fractionated from total RNA of 8-h treated aleurone layers by oligo(dT)–cellulose chromato-

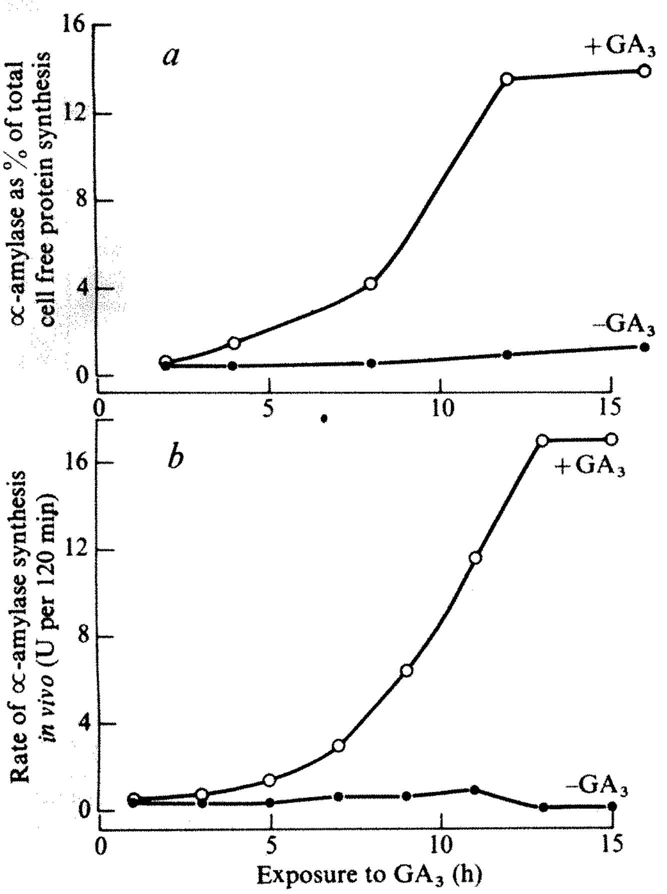


Fig. 1 Fluorographs of SDS–polyacrylamide gel electrophoresis of cell-free translation products of RNA isolated from aleurone layers 8 h after the beginning of hormone treatment. Aleurone layers were prepared from barley (var. Himalaya) half-seeds and incubated as before[4], at 25 °C in medium containing 10^{-2} M $CaCl_2$ and 10^{-6} M GA_3 where required. Total RNA was prepared by the following modification of the method of Click and Hackett[25]. The layers were ground in 10 ml of buffer containing 0.1 M sodium glycinate, *p*H 9.5, 0.01 M EDTA, 0.1 M sodium chloride and 1% SDS, with 1% insoluble polyvinylpyrrolidone (L. Taiz, personal communication). After centrifugation at 10,000*g* for 10 min an equal volume of phenol–chloroform (1 : 1) was added to the supernatant and the aqueous phase was extracted for 5 min while being shaken. The phases were separated, the phenol phase was shaken for a further 5 min with 5 ml of the same buffer and the combined aqueous phases were then extracted with 5 ml of phenol–chloroform. The RNA in the aqueous phase was precipitated by addition of 2.5 volumes of ethanol and stored overnight at −20 °C. It was then pelleted by centrifugation, redissolved in 3 ml of 0.5 M sodium acetate, *p*H 5.8 and reprecipitated with 0.4 ml of 1% acetyltrimethylammonium bromide[27]. The precipitate was recovered by centrifugation and washed with 0.1 M sodium acetate in 70% ethanol and finally with 3 M sodium acetate, 5 mM EDTA, *p*H 5.0. The RNA was then used directly or fractionated on oligo(dT)–cellulose as before[28] except that SDS (0.1%) was included in the buffer. After precipitation, the RNA was used to program the wheat embryo cell-free protein synthesising system[21] as modified[24]. ^{35}S-methionine (50 Ci mol^{-1}, Amersham) was the source of radioactive amino acid. The reaction was stopped after 90 min at 25 °C. The ribosomes were removed by centrifugation at 30,000 r.p.m. for 60 min and the soluble proteins were either analysed on SDS–polyacrylamide gels[23] (14%) in the presence of 2-mercaptoethanol with the modifications specified[24] or subjected to immunoprecipitation followed by SDS–polyacrylamide gel electrophoresis of the antibody precipitates. Sliced and stained gels were prepared for fluorography according to Bonner and Laskey[26]. The dried gels were exposed to Kodak X-OMAT X-ray film for 7 d at −80 °C. Immunoprecipitation was carried out on the proteins of the cell-free system from which the ribosomes had been removed. Antiserum (10 μl) produced in rabbits against purified α-amylase[31,32] was added to each reaction mixture and incubated at 25 °C for 60 min. Sufficient goat anti-rabbit gammaglobulin serum (provided by Dr David Kemp) was then added to precipitate all the rabbit antibodies and the mixture was incubated for 60 min at 25 °C and then overnight at 4 °C. The precipitates were collected by centrifugation through sucrose cushions and washed twice with saline–detergent as described[33] and modified[24]. The immunoprecipitates were then analysed on SDS–polyacrylamide gels and prepared as described above. Cell-free translation products of total RNA: *a*, $-GA_3$; *b*, $+GA_3$. Products of poly(A)-RNA: *c*, $-GA_3$; *d*, $+GA_3$. Specific anti-α-amylase immunoprecipitates of translation products of total RNA: *e*, $-GA_3$; *f*, $+GA_3$. The numbers refer to the molecular weights ($\times 10^{-3}$) of the markers, bovine serum albumin (molecular weight 68,000), ovalbumin (molecular weight 45,000), chymotrypsinogen (molecular weight 25,000) and cytochrome *c* (molecular weight 12,400). The arrow denotes the position to which authentic α-amylase migrated, and 0 refers to the origin.

graphy[28] and was used to program the cell-free system. This step resulted in a tenfold increase in template specific activity. The patterns of cell-free translation products were identical with those obtained with total RNA (Fig. 1*c* and *d* compared with Fig. 1*a* and *b*). Our observations thus provide further evidence[29,30], that the poly(A)-RNA of plants includes RNA with messenger activity.

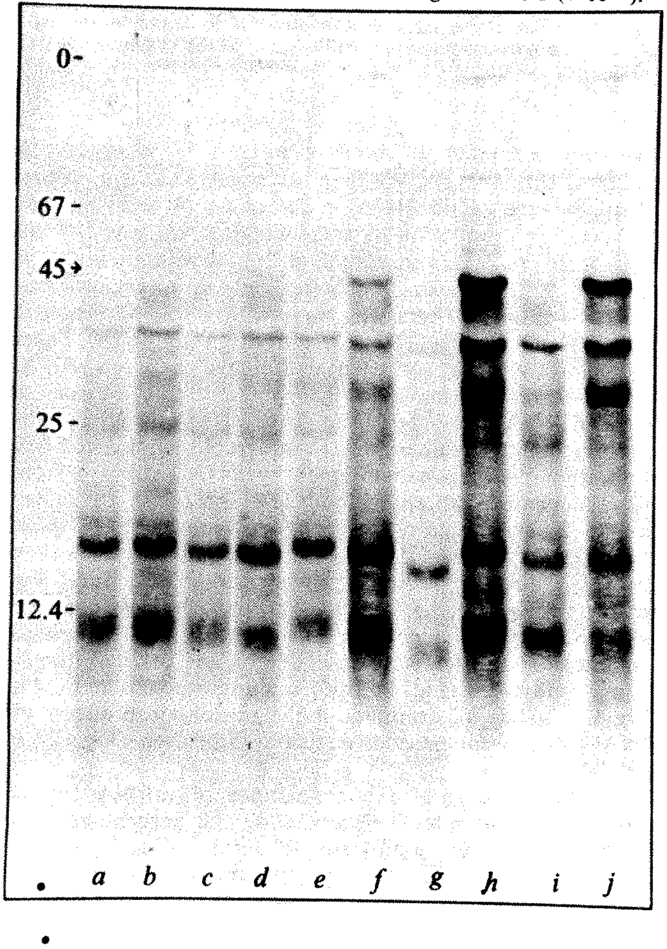
Evidence for the identification of the 45,000-dalton polypeptide synthesised *in vitro* was provided by immunoprecipitation with monospecific antibodies produced in rabbits against barley α-amylase[31,32]. The double antibody technique was used to precipitate the anti-α-amylase[22]. Although the unfractionated translation products of total RNA were complex (Fig. 1*a* and *b*) the immunoprecipitation step yielded a single polypeptide which coelectrophoresed with or was slightly larger than authentic α-amylase run on the same gel and stained with Coomassie blue (Fig. 1*f*). Sufficient material was not available for tryptic fingerprint analysis and it was therefore not certain whether the cell-free product was identical with the authentic protein or whether it contained some extra amino acids at either the NH_2– or –COOH terminals. A study of the cell-free synthesis of immunoglobulin light chain showed that the *in vitro* product was larger than the polypeptide found *in vivo*[34]. RNA isolated from aleurone layers incubated without hormone did not direct the cell-free synthesis of detectable α-amylase as determined by immunoprecipitation (Fig. 1*e*).

Based on the above evidence, we tentatively concluded that GA_3 increased the level of mRNA for α-amylase in barley aleurone layers. We then investigated the time course of appearance of the RNA in the tissue by assaying with the cell-free translation system. Total RNA was isolated from aleurone layers incubated with and without hormone for 2, 4, 8, 12 and 16 h and was used to direct cell-free protein synthesis. There was two- to threefold variation in template efficiency of RNA from batch to batch. There was, however, no consistent difference between RNAs isolated from hormone-treated and untreated tissue or between RNAs extracted from tissue after exposure to the hormone for different times. Fluorographs of the cell-free translation products programmed by RNA extracted from tissue incubated with and without GA_3 for 2–16 h are shown in Fig. 2. The range of polypeptides was similar to those shown in Fig. 1*a* and *b* and the major difference between the RNA-directed products of hormone-treated and untreated tissue were the 35,000-dalton polypeptide(s) and the polypeptide earlier identified as α-amylase. The increased amount of the latter was detectable on the original fluorographs within 2 h (Fig. 2*b*) and was quite apparent by 4 h (Fig. 2*d*) and in all examples of the translation products of RNA from hormone-treated tissue thereafter (Fig. 2*f*, *h* and *j*).

The time course of the appearance of mRNA for α-amylase was estimated by calculating the percentage contribution made by α-amylase to total cell-free protein synthesis. Autoradiographs were prepared by exposing X-ray film to the dried gels, similar to those in Fig. 2, for different times and the profiles were scanned by microdensitometry[35]. α-Amylase made an increasing contribution to protein synthesis when the *in vitro* system was programmed with RNA extracted from tissue incubated with hormone for increasing periods up to 12 h (Fig. 3*a*). GA$_3$ treatment for 16 h did not stimulate further the production of template RNA for α-amylase (Fig. 3*a*). The *in vivo* production of total α-amylase in response to the hormone is shown in Fig. 3*b* plotted as the rate of α-amylase synthesis rather than as total enzyme level. There is a positive correlation between the rate of α-amylase synthesis *in vivo* and the level of mRNA for α-amylase as assayed by the cell-free system. Investigation of the stimulation of ovalbumin synthesis in chick oviduct by oestrogen has shown that the level of mRNA for ovalbumin increases in response to the hormone[33,36,37], and that the rate of synthesis of the oviduct protein is controlled chiefly by the concentration of cellular mRNA[38].

Our data show that GA$_3$ increases the level of translatable mRNA for α-amylase, but we cannot distinguish between the following three mechanisms whereby the level might be increased: (1) a decreased rate of mRNA degradation; (2) enhancement of the translational capacity of mRNA for α-amylase through processing, activation or release of RNA from some inactive form (this possibility is relatively unlikely since total deproteinised RNA was used, eliminating the possibility that mRNP or other protein-masked forms of RNA were involved); and (3) synthesis of new mRNA molecules. Our results taken together with the fact that GA$_3$ stimulated poly(A)-RNA synthesis[18,20], although not eliminating an effect on degradation, provide correlative evidence that GA$_3$ promotes the synthesis of mRNA for α-amylase.

Fig. 2 Fluorographs of SDS–polyacrylamide gel electrophoresis of the cell-free translation products of RNA isolated from aleurone layers at various times after hormone treatment. Total RNA was extracted from aleurone layers (as described in the legend to Fig. 1) after 2, 4, 8, 12 and 16 h in buffer or buffer plus GA$_3$ and used to program the cell-free protein synthesising system. *a*, *c*, *e*, *g* and *i*, Translation products of total RNA from aleurone layers incubated without GA$_3$ for 2, 4, 8, 12 and 16 h respectively; *b*, *d*, *f*, *h* and *j*, translation products of total RNA from aleurone layers incubated with 10^{-6} M GA$_3$ for 2, 4, 8, 12 and 16 h respectively. The arrow denotes position of α-amylase and the numbers refer to molecular weight markers (×10^{-3}).



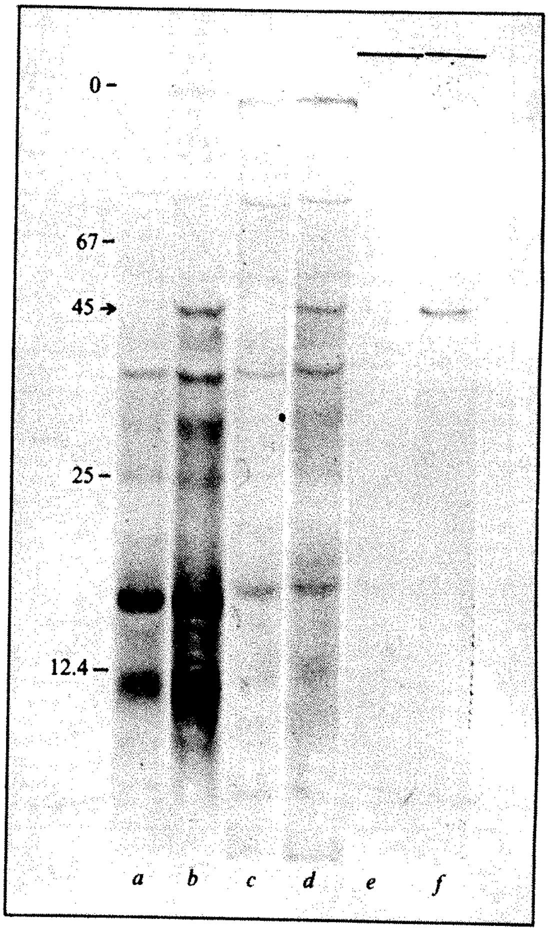


Fig. 3 The increase with time in the level of translatable mRNA for α-amylase and the increase in the rate of synthesis of the enzyme *in vivo* in response to GA$_3$ treatment. *a*, α-Amylase synthesised by the cell-free system was estimated from densitometric scans of autoradiographs of gels which had been in contact with X-ray film for different times. The gels were scanned using a Joyce–Loebl microdensitometer and the contribution made by the α-amylase peak to total protein synthesised was calculated by weight[35]. *b*, The rate of α-amylase synthesis *in vivo* was calculated as the increment in enzyme level (units of α-amylase) during successive 2-h intervals following hormone treatment. Total α-amylase activity was assayed using replicates of 10 aleurone layers as described[4]. U, Units of α-amylase.

We thank our colleagues for reading the manuscript and Dr P. B. Goodwin for advice. The assistance of Lois Moore, Marliese Caldersmith and Rosemary Metcalf is acknowledged.

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Received December 15, 1975; accepted January 26, 1976.

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Short-chain fatty acids as inhibitors of gibberellin-induced amylolysis in barley endosperm

ABSCISIC acid (ABA) is the only plant growth substance so far reported[1,2] to inhibit gibberellin(GA)-induced amylolyis in barley half seeds or α-amylase production in barley aleurone layers: the former physiological response has been used as a bioassay for ABA[3,4]. But the isolation procedures normally used to extract ABA from plant tissue also extract certain short-chain fatty acids[5]. We now present evidence that these naturally occurring short-chain acids[5] are powerful inhibitors of GA-induced amylolysis in barley endosperm.

Embryo-free half seeds were prepared[6] from husked barley (cultivar Ymer) and GA-induced starch hydrolysis was assayed by measuring[7,8] the amount of reducing sugar released into the medium (1.05 ml) by pairs of half endosperms incubated in the presence and absence of GA (80% GA_3 20% GA_1; 7.2×10^{-7} M) (B and G in Fig. 1). Buffering of the GA solution to *p*H 4.8 with citrate (10^{-3} M) did not alter the response to GA (C in Fig. 1). The effect of straight-chain carboxylic acids was assessed by replacing the citrate buffer with acetate (C_2), propionate (C_3), *n*-butanoate (C_4), *n*-pentanoate (C_5) . . . . . *n*-undecanoate (C_{11}) buffers[9] (10^{-3} M, *p*H 4.8) and the results are shown in Fig. 1. All these acids inhibit amylolysis to some extent; even acetate causes a 30% reduction in sugar release—a phenomenon first noted by Paleg[10]. The exceptional efficacy of pentanoic (C_5) and nonanoic (C_9) acids in overcoming the effect of GA is superimposed on a general increase in inhibition from C_1 to C_9 with a subsequent decrease at longer chain lengths.

Dose–response data for ABA and the four most inhibitory fatty acids (C_5, C_7, C_8 and C_9) are presented in Fig. 2. Clearly nonanoic aicd (C_9) is the most effective of the fatty acids but the most striking feature of Fig. 2 is the difference between the dose–response curves for ABA on the one hand and the fatty acids on the other. Inhibition by ABA is total at 10^{-4} M, virtually zero at 10^{-8} M and at intermediate concentrations the response is proportional to the logarithm of the concentration. On the other hand, inhibition by fatty acids (for example, C_5 and C_9 in Fig. 2) is total at 10^{-3} M but nonexistent at 10^{-5} M. Stated differently, inhibition by ABA is a finely graded phenomenon, infinitely variable over a 10,000-fold concentration range whereas inhibition by fatty acids is more akin to a threshold effect. We have detected nonanoic acid in ungerminated barley seed and if such fatty acids are true plant growth substances—and evidence is accumulating rapidly that they are—the threshold effect could be important in the regulation of "all or nothing" phenomena such as germination or release from dormancy.

In Glasgow and in our laboratories in Stirling, reports are being prepared of positive correlations between fatty

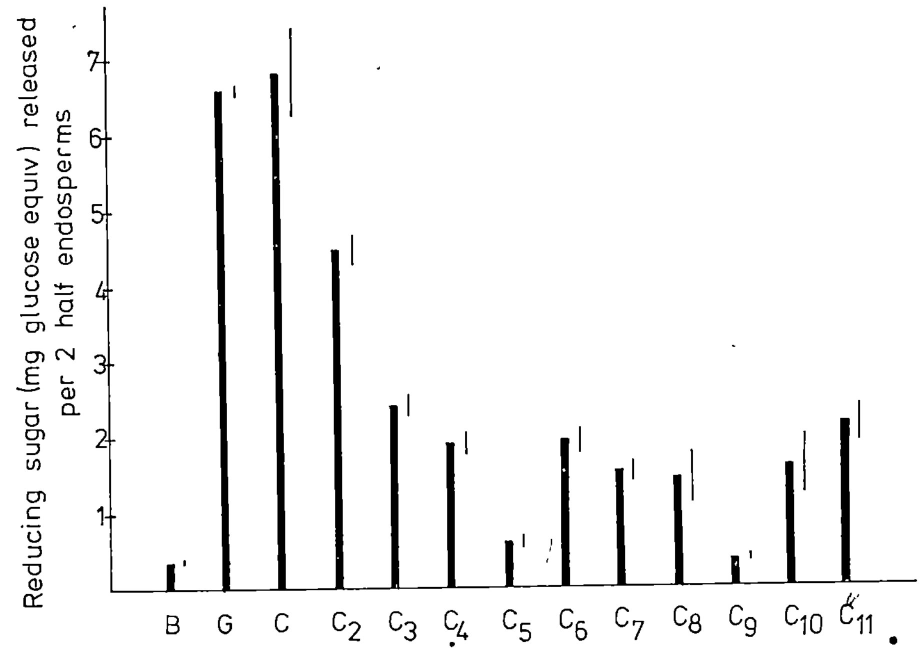

Fig. 1 Influence of normal, short-chain fatty acids on GA-induced amylolysis in barley endosperm. The vertical bars represent the amount (± s.e.m.) of reducing sugar released into the incubation medium by embryo-free half endosperms incubated in pairs (four replicates) for 42 h at 26 °C in the presence of water alone (B); 4.2×10^{-7} M gibberellin in water (G); 4.2×10^{-7} M gibberellin in 10^{-3} M citrate buffer *p*H 4.8 (C); 4.7×10^{-7} M gibberellin in 10^{-3} M acetate (C_2), propionate (C_3), butanoate (C_4) . . . undecanoate (C_{11}) buffers, all at *p*H 4.8.

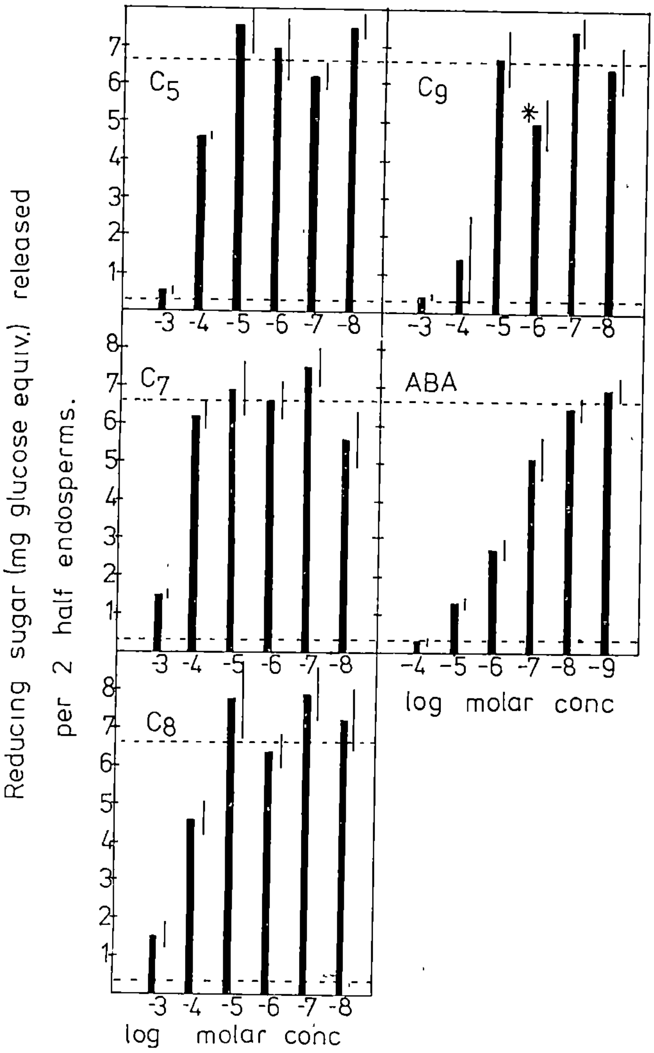

Fig. 2 Concentration dependence of fatty acid and ABA inhibition of GA-induced amylolysis in barley endosperm. Vertical bars represent reducing sugar released (see caption to Fig. 1) in presence of pentanoic (C_5), heptanoic (C_7), octanoic (C_8) and nonanoic (C_9) acids and ABA in the concentrations stated. All incubations were carried out at a constant GA concentration of 4.2×10^{-7} M, and *p*H 4.8. — — —, Mean blank value, that is, reducing sugar released in the absence of GA and inhibitors. - - - - - -, Mean control value, that is, reducing, sugar released in the presence of GA and absence of inhibitor. *Not significantly different from control value at $P<0.10$.

acid levels and depth of dormancy in oat; aleurone layer activity in fenugreek seed; the mechanism of stomatal functioning, and leaf abscission and ethylene production. The *in vitro* inhibition of amylolysis described here represents yet another phenomenon which may have considerable physiological relevance.

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Received December 15, 1975; accepted January 22, 1976

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DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA

Infection of fibroblasts by avian sarcoma virus (ASV) leads to neoplastic transformation of the host cell. Genetic analyses have implicated specific viral genes in the transforming process[1-4], and recent results suggest that a single viral gene is responsible[4]. Normal chicken cells contain DNA homologous to part of the ASV genome[5-8]; moreover, embryonic fibroblasts from certain strains of chickens can produce low titres of infectious type C viruses either spontaneously[9] or in response to various inducing agents[10]. None of the viruses obtained from normal chicken cells, however, can transform fibroblasts, and results with molecular hybridisation indicate that the nucleotide sequences responsible for transformation by ASV are not part of the genetic complement of the normal cell[11]. We demonstrate here that the DNA of normal chicken cells contains nucleotide sequences closely related to at least a portion of the transforming gene(s) of ASV; in addition, we have found that similar sequences are widely distributed among DNA of avian species and that they have diverged roughly according to phylogenetic distances among the species. Our data are relevant to current hypotheses of the origin of the genomes of RNA tumour viruses[12] and the potential role of these genomes in oncogenesis[13].

We have prepared radioactive DNA ($\text{cDNA}_{\text{sarc}}$) complementary to nucleotide sequences which represent most or all of the viral gene(s) required for transformation of fibroblasts by ASV[14]. Our procedure to isolate $\text{cDNA}_{\text{sarc}}$ exploited the existence of deletion mutants of ASV which lack 10–20% of the viral genome (transformation defective, or td viruses)[11,15-17]; results of genetic analyses indicate that the deleted nucleotide sequences include part or all of the gene(s) responsible for oncogenesis and cellular transformation[4,14]. In our procedure the genome of the Prague-C strain (Pr-C) of ASV was transcribed into complementary DNA by endogenous RNA-directed DNA polymerase activity; we then used molecular hybridisation to select DNA specific for the region missing from the genome of the td deletion mutants. The preparation of $\text{cDNA}_{\text{sarc}}$ used was a virtually uniform transcript from about 16% of the Pr-C ASV genome[14], a region equivalent in size to the entire deletion in the strain of td virus used in our experiments[11,15-17]. Since the unit genome of ASV contains about 10,000 nucleotides[18,19], the genetic complexity of $\text{cDNA}_{\text{sarc}}$ is about 1,600 nucleotides, sufficient to represent an entire cistron. Nucleotide sequences homologous to $\text{cDNA}_{\text{sarc}}$ seem to be ubiquitous in the genomes of ASVs, but are not present in the genomes of avian leukosis viruses (including the endogenous chicken virus, RAV-0) or sarcoma-leukosis viruses from other species[14].

DNAs from several avian species (chicken, turkey, quail, duck and emu) contain nucleotide sequences which can anneal with $\text{cDNA}_{\text{sarc}}$ (Fig. 1 and Table 1). In contrast, we detected no homology between $\text{cDNA}_{\text{sarc}}$ and DNA from mammals (mouse and calf thymus; Table 1). The kinetics of the reactions between $\text{cDNA}_{\text{sarc}}$ and DNAs from chicken, quail and duck (Fig. 1*a*, *c* and *d*) were similar to the kinetics for the reassociation of unique nucleotide sequences in avian DNAs (C_0t about 1,000 mol s^{-1})[20]; thus, nucleotide sequences homologous to $\text{cDNA}_{\text{sarc}}$ are present as single (or a few) copies in each haploid complement of the avian DNAs tested. This conclusion was substantiated by measuring in a single reaction

Table 1 Homology between cDNA$_{\mathrm{sarc}}$ and normal DNAs

Assay	Hybridisation conditions [Na$^+$]	Temperature	Chicken	Quail	Turkey	Duck	Emu	Mouse	Calf
S1	0.9 M	68°	52%	46%	48%	45%	24%	<2%	<2%
HAP	0.9 M	68°					36%		<5%
HAP	1.5 M	59°					54%		

Extent of reaction between cDNA$_{\mathrm{sarc}}$ and DNA from: Chicken, Quail, Turkey, Duck, Emu, Mouse, Calf.

DNA was extracted from 10–11-d-old embryos of chickens, ducks and quails, 3-d-old mice (strain RIII), livers of adult turkeys, liver and heart of a 22-d-old emu, and calf thymus. Reaction mixtures contining denatured DNA (8 mg ml^{-1}) and ^{3}H-cDNA$_{\mathrm{sarc}}$ (0.32 ng ml^{-1}, 7,000 c.p.m. ml^{-1}) in a final volume of 0.3 ml were incubated at either 59 or 68 °C for 48 h. Samples incubated at 59 °C were in 1.5 M NaCl (final $C_0t = 40{,}000$), those incubated at 68 °C were in 0 9 M NaCl (final $C_0t = 32{,}000$); all reactions also continued 0.001 M EDTA–0.02 M Tris-HCl, *p*H 7.4. Duplex formation was measured by either hydrolysis with S1 nuclease[28] (in 0.3 M NaCl at 50 °C) or fractionation on hydroxyapatite (HAP) (samples adsorbed in 0.14 M sodium phosphate at 50 °C).

mixture the rates of reassociation between chicken DNA and both cDNA$_{\mathrm{sarc}}$ (labelled with ^{3}H) and unique nucleotide sequences purified from chicken DNA (labelled with ^{14}C) (Fig. 1*b*); the rates were similar for both labelled DNAs.

The extent of duplex formation with cDNA$_{\mathrm{sarc}}$ varied with the amount of chicken DNA used in the reactions (Fig. 1*a* and *b*), as expected in reactions where identical labelled and unlabelled DNA strands are competing for unlabelled complementary strands, and the reactions were incomplete (about 50%) at the highest value of C_0t (Fig. 1*a*, *c* and *d* and Table 1). Nevertheless, we believe that most or all of the nucleotide sequences of cDNA$_{\mathrm{sarc}}$ are present in the DNA of quail since cDNA$_{\mathrm{sarc}}$ anneals completely with RNA from certain quail cells (unpublished results of ourselves and C. Moscovici). Moreover, the rates and extents of annealing between cDNA$_{\mathrm{sarc}}$ and DNA from chicken, duck and turkey are approximately the same as the rate and extent of annealing between cDNA$_{\mathrm{sarc}}$ and quail DNA (Table 1 and Fig. 1). Thus, it is likely that most or all of the nucleotide sequences of cDNA$_{\mathrm{sarc}}$ are present in the DNA of all these birds.

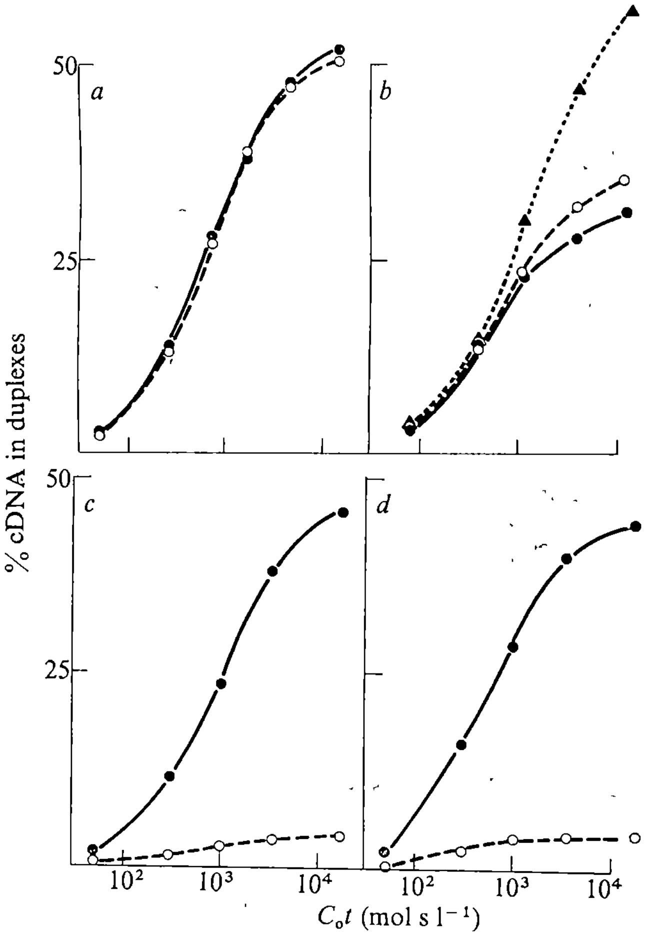

Fig. 1 Annealing of cDNA$_{\mathrm{sarc}}$ to normal avian DNAs. DNA was prepared from 10–11-d-old embryos[7], denatured and incubated with ^{3}H-cDNA$_{\mathrm{sarc}}$ and ^{32}P-cDNA$_{\mathrm{B77}}$ in 0.6 M NaCl-0.001 EDTA–0.02 M Tris-HCl, *p*H 7.4 at 68 °C for 0 1 to 32 h. Samples (0.1–0.2 ml) were withdrawn at different times and the extent of duplex formation with cDNA$_{\mathrm{sarc}}$ and cDNA$_{\mathrm{B77}}$ was measured by hydrolysis with the single-strand specific nuclease, S1 (ref. 28). The preparation of cDNA$_{\mathrm{sarc}}$ and cDNA$_{\mathrm{B77}}$ has been described elsewhere[14] and is outlined in the text. Unique sequence chicken DNA was prepared by reannealing denatured ^{14}C-chicken DNA to a C_0t of 500; unique sequence DNA was eluted from a hydroxyapatite column at 60 °C in 0 14 M phosphate buffer[20]. *a*, Chicken DNA (9 mg ml^{-1}) with ^{3}H-cDNA$_{\mathrm{sarc}}$ (0.5 ng ml^{-1}, 10,000 c.p.m. ml^{-1}) (●) and ^{32}P-cDNA$_{\mathrm{B77}}$ (0.3 ng ml^{-1}, 10,000 c.p.m. ml^{-1}) (○). *b*, Chicken DNA (5 mg ml^{-1}) with ^{3}H-cDNA$_{\mathrm{sarc}}$ (0.45 ng ml^{-1}, 9,000 cpm ml^{-1}) (●); ^{32}P-cDNA$_{\mathrm{B77}}$ (0.5 ng ml^{-1}, 6,000 c.p.m. ml^{-1}) (○), and ^{14}C-chicken unique sequence DNA (▲). *c*, Quail DNA (9 mg ml^{-1}) with ^{3}H-cDNA$_{\mathrm{sarc}}$ and ^{32}P-cDNA$_{\mathrm{B77}}$ as in *a*. *d*, Duck DNA (9 mg ml^{-1}) with ^{3}H-cDNA$_{\mathrm{sarc}}$ and ^{32}P-cDNA$_{\mathrm{B77}}$ as in *a*.

The extent of the reaction between cDNA$_{\mathrm{sarc}}$ and DNA from emu, a relatively primitive Australian bird, was limited (24%) when tested by hydrolysis with a single strand-specific nuclease (Table 1); the extent of the reaction was greater when analysed on hydroxyapatite, a less stringent procedure than the nuclease test (Table 1), and was further augmented when the annealings were performed in conditions which facilitate pairing of partially matched nucleotide sequences (1.5 M NaCl, 59 °C, ref. 21) (Table 1). These date indicate that the nucleotide sequences homologous to cDNA$_{\mathrm{sarc}}$ in emu DNA are substantially diverged from the homologous sequences in the other avian DNAs; we have obtained further data to sustain this conclusion by analysing the thermal stability of the duplexes formed between cDNA$_{\mathrm{sarc}}$ and various avian DNAs (see below, Table 2 and Fig. 2).

Avian DNAs were also tested with ^{32}P-labelled single-stranded DNA, complementary to the RNA genome of the B77 strain of ASV (cDNA$_{\mathrm{B77}}$), synthesised with detergent-disrupted virions, and purified as described previously[14] The virus used to prepare cDNA$_{\mathrm{B77}}$ consisted mainly of td variants (about 90% of the particles; unpublished observations). Consequently, DNA synthesised with the virus was deficient in nucleotide sequences homologous to cDNA$_{\mathrm{sarc}}$ and served principally to detect other portions of the ASV genome. A substantial fraction (about 50%) of cDNA$_{\mathrm{B77}}$ reacted with normal chicken DNA, but there was little or no reaction with quail, duck, turkey and emu DNAs (Fig. 1 and unpublished data); these results conform to previous reports[20,22,23]. We conclude that the DNAs from widely divergent avian species all contain nucleotide sequences which are at least partially related to transforming gene(s) of ASV, whereas only chicken DNA has appreciable homology with the remainder of the ASV genome.

The relatedness of DNA sequences homologous to cDNA$_{\mathrm{sarc}}$ in different avian species was analysed by denaturing duplexes formed between cDNA$_{\mathrm{sarc}}$ and normal avian DNAs (Fig. 2 and Table 2). In addition, we denatured duplexes between cDNA$_{\mathrm{sarc}}$ and DNA from XC cells (rat cells transformed by Pr-C ASV) to test completely matched duplexes containing cDNA$_{\mathrm{sarc}}$ sequences. The mammalian DNAs we have tested (calf and mouse) contain no nucleotide sequences homologous to cDNA$_{\mathrm{sarc}}$ before in-

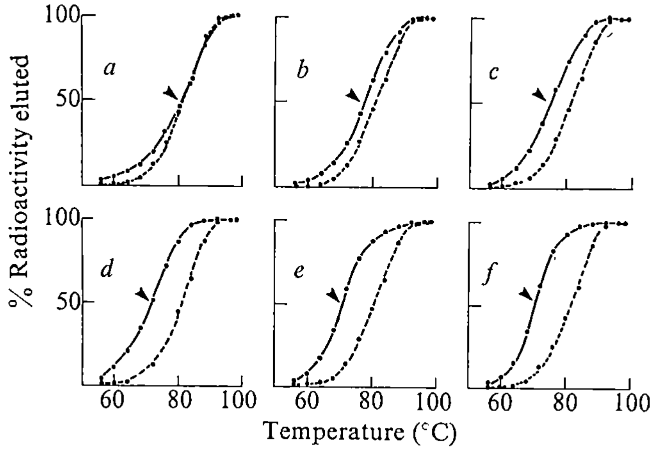

Fig. 2 Thermal denaturation of duplexes formed between cDNA$_{sarc}$ and cellular DNAs. DNA was extracted from XC cells grown in culture, from 10–11-d-old embryos of chickens, ducks and quails, from the livers of adult turkeys, and from liver and heart of a 22-d-old emu. Denatured DNAs (0.5 mg) were incubated with ^{3}H-cDNA$_{sarc}$ (0.25 ng, 5,000 c.p.m) in 0.1 ml of 0.9 M NaCl-0.001 M EDTA-0.02 M Tris-HCl, *p*H 7.4, for 100 h at 68 °C, final C_0t = 40,000. In a separate reaction, ^{32}P-cDNA$_{B77}$ (2.5 ng, 100,000 c.p.m.) was annealed with 7 mg of denatured chicken DNA (final C_0t = 80,000). Samples (0.4 mg of chicken DNA and 6,000 c.p.m. of cDNA$_{B77}$) of this reaction were added to the DNAs which had been annealed with cDNA$_{sarc}$. The mixtures were then passed through a column of hydroxyapatite (3 ml packed vol) in 0.12 M sodium phosphate, *p*H 6.8, at 56 °C; in these conditions, stable duplexes bind to the hydroxyapatite, whereas single-stranded DNA does not. The columns were then washed continuously with 0.12 M sodium phosphate (0.4 ml min^{-1}) while the temperature of the column was raised in increments of 4 °C every 10-min. Fractions (4 ml) were collected and analysed for acid-precipitable radioactivity. ●——●, Duplexes with cDNA$_{sarc}$; ●- - - -●, duplexes with cDNA$_{B77}$ and chicken DNA (internal standard). *a*, Provirus (XC); *b*, chicken; *c*, quail; *d*, turkey; *e*, duck; *f*, emu.

fection by ASV; consequently, the DNA homologous to cDNA$_{sarc}$ in rat cells after infection presumably represents nucleotide sequences contained entirely in recently integrated provirus for ASV rather than related sequences present in the DNA of uninfected cells. Denaturation was standardised internally by adding duplexes formed in a separate annealing reaction between normal chicken DNA and ^{32}P-labelled cDNA$_{B77}$; these duplexes denatured with a T_m of 81±1 °C.

Duplexes between cDNA$_{sarc}$ and DNA from XC cells had the highest T_m (81 °C; Table 2), as expected for completely matched complementary nucleotide sequences. Duplexes with chicken DNA were slightly less stable (T_m=77 °C); the nucleotide sequences of cDNA$_{sarc}$ in the ASV genome are apparently diverged from the homologous sequences in the avian host. The reduction in the T_m is consistent with about 3% mismatching of bases[24]. The stability of duplexes with other avian DNAs (T_m=70–74 °C) decreased roughly in accord with phylogenetic distances among the species tested (Table 2); the T_ms suggest 5–8% mismatching of base pairs[24]. We made no effort in these tests to obtain maximum duplex formation with cDNA$_{sarc}$, and the reactions with quail, turkey, duck and emu were relatively limited in extent (15–37%). In other experiments where at least 50% of cDNA$_{sarc}$ was annealed into duplexes with chicken, quail and duck DNAs, we observed T_ms similar to those given in Table 2. cDNA$_{sarc}$ seems to represent nucleotide sequences which arose and diverged during the course of avian speciation.

We have shown previously that the nucleotide sequences of cDNA$_{sarc}$ include genetic information required for transformation of fibroblasts by ASV[14]; the data reported here indicate that similar or partially related information is present in the genome of the normal chicken cell and widely distributed among the avian species. We suggest that part or all of the transforming gene(s) of ASV was derived from the chicken genome or a species closely related to chicken, either by a process akin to transduction[25] or by other events, including recombination, which are alleged to have generated type C viruses from normal cellular genes[12]. If the entire ASV genome originated from cellular genes[12], then nucleotide sequences in the transforming gene(s) have been conserved relative to other viral genes, because the nucleotide sequences of cDNA$_{sarc}$ are the only portion of the ASV genome which we can detect in DNA from birds other than chickens. We cannot exclude, however, the possibility that viral genes other than those represented by cDNA$_{sarc}$ have been introduced into the germ line of chickens after speciation. The sequences homologous to cDNA$_{sarc}$ in the genome of ASV are slightly diverged from the analogous sequences in chicken genome; this could be the consequence of either the process which generated viral genes from cellular genes[12] or mutations during the course of repeated viral propagation.

Others have reported evidence concerning the genetic divergence during possible spread of unidentified portions of type C viral genomes during evolution[26,27] and the apparent transduction of cellular nucleotide sequences by type C viruses[25], but our study provides the first data on the origins of a genetically identified set of viral nucleotide sequences. It should be possible to carry out similar studies for at least one other gene of ASV (the gene coding for the type-specific glycoprotein), using techniques similar to those reported here.

Neiman and his colleagues have reported that the hybridisation of ASV 70S RNA to normal chicken DNA was competed completely by RNA from non-transforming viruses[11]. They therefore concluded that the genes responsible for transformation by ASV were present in the avian genome only after viral infection. We cannot explain the discrepancy between their results and ours.

We anticipate that cellular DNA homologous to cDNA$_{sarc}$ serves some function which accounts for its conservation during avian speciation. The nucleotide sequences which anneal with cDNA$_{sarc}$ are part of the

Table 2 Thermal stabilities of duplexes between cDNA$_{sarc}$ and normal DNAs

DNA	%cDNA$_{sarc}$ in duplexes	T_m	ΔT_m	Phylogenetic distance from chicken (Myr)
Provirus (XC)	56	81	0	
Chicken	52	77	−4	0
Quail	37	74	−7	35–40
Turkey	30	72	−9	40
Duck	16	71	−10	80
Emu	15	70	−11	100

Denatured DNAs (0.5 mg) were annealed with ^{3}H-cDNA$_{sarc}$ (0.25 ng, 5,000 c.p.m.), adsorbed to hydroxyapatite in 0.12 M sodium phosphate at 56 °C, and denatured with a thermal gradient, all as described for Fig. 2. Duplexes between ^{32}P-cDNA$_{B77}$ and normal chicken DNA, included in each analysis as an internal standard, denatured with T_m = 81 ±1 °C. The estimates of phylogenetic distance, deduced from fossil records and antigenic relationships among proteins[29], were provided by Professor Allan Wilson (personal communication).

unique fraction of cellular DNA and could represent either structural or regulatory genes. But the function of those sequences is unknown. We are testing the possibilities that they are involved in the normal regulation of cell growth and development or in the transformation of cell behaviour by physical, chemical or viral agents.

We thank William E. Meeker of Sacramento Zoo for assistance in obtaining the emu, Karen Smith and Jean Jackson for technical assistance, and H. Temin and L. Levintow for editorial advice. This work was supported by grants from the US Public Health Services and the American Cancer Society, and a contract within the Virus Cancer Program of the National Cancer Institute. D.S. was supported by CNRS and H.E.V. is a recipient of a research career development award from the National Cancer Institute.

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Received November 10, 1975; accepted January 2, 1976.

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Raman spectra of transfer RNAs with ultraviolet lasers

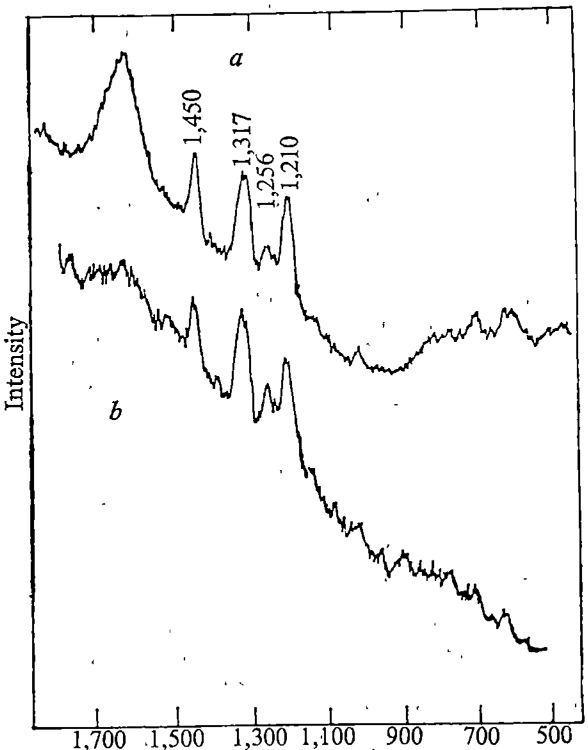
WE suggest here a new use for Raman spectroscopy in structural studies of nucleic acids. We demonstrate that an enhanced vibrational Raman spectrum of a certain limited portion of an amino acid transfer RNA (tRNA) macromolecule can be observed, if a proper ultraviolet laser beam is chosen for excitation.

Until now, Raman spectra of tRNAs have been observed with laser beams of visible light[1–6]. They all show a number of Raman lines characteristic of the four common bases, namely, adenine, uracil, guanine, and cytosine. For such observations concentrated solutions (usually about 5 %) or considerable amounts (10 mg) of tRNA are required. Some of the minor constituent nucleosides in tRNAs have strong absorption bands in the 300–350-nm region, whereas the common bases have them at 260 nm. Using the correct ultraviolet laser, therefore, one would expect to observe resonance Raman effects of minor nucleoside bases. Such effects would be valuable, first, because they would provide information on one particular base residue of the 70–80 bases in tRNA and second, because this could be done with much smaller amount samples of tRNA this usual.

We have observed a Raman spectrum of formylmethionine tRNA of *Escherichia coli* with the 363.8-nm beam of an argon ion laser (Coherent Radiation 52 GA). The power of the laser beam was 5 mW at the sample. The tRNA concentration of the sample solution was only 0.3%, in cacodylate buffer, *p*H 7.2. The total volume of the sample solution used was 0.2 ml, contained in a cylindrical cell of silica glass. A JRS-U1 Raman spectrophotometer (Japan Electron Optics) was used. The recorded curve is shown in Fig. 1*a*. The curve can be reproduced more than ten times from one sample solution. The aminoacylation activity of the tRNA remained undegraded after 5 h irradiation with the ultraviolet laser beam. When the wavelength of the laser beam was changed from 363.8 to 351.1 nm, almost the same Raman spectrum was observed.

All of the observed Raman lines are attributable to the 4-thiouridine residue (at position 8) subjected to photochemical modification (probably cross linking[7,8] with the cytidine residue at position 13 of the tRNA). This can be concluded because, first, at that concentration and with that laser power, on the basis of the available data, none of the Raman lines of the common base residues can be expected to appear. Moreover, none of the

Fig. 1 Raman spectra excited by the 3,638 Å beam of an argon ion laser. *a*, tRNA^{fMet} from *E. coli;* concentration, 0.3 %; solvent, 10^{-1} M NaCl+10^{-2} M Na-cacodylate (*p*H 7.2)+10^{-3} M MgCl$_2$. *b*, tRNA^{Gly} from *E. coli;* concentration, 0.3 %; solvent as in *a*.



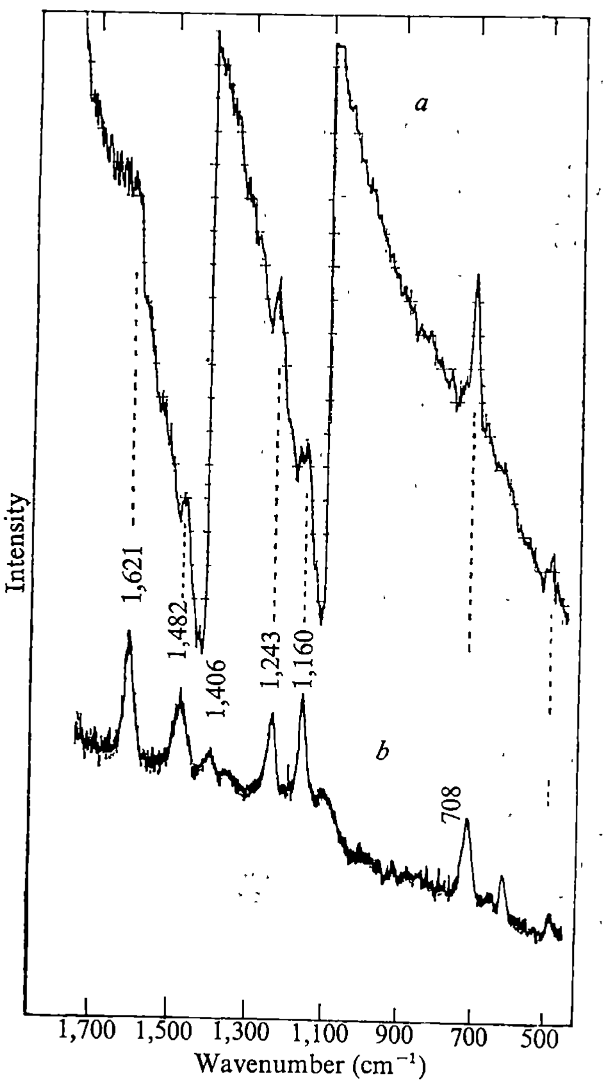


Fig. 2 Raman spectra excited by the 3,638 Å beam of an argon ion laser. *a*, tRNA^{Tyr} (2) from *E. coli*; concentration, 1 %; solvent, 10^{-1} M NaCl+10^{-2} M Na-cacodylate (*p*H 7.2),+10^{-3} M $MgCl_2$. *b*, 4-Thiouridine; concentration, 10^{-4} M; solvent, 10^{-2} M Na-cacodylate, *p*H 7.2; observed with a rotating cell.

observed lines has the same Raman frequency as the common base residues. Second, 4-thiouridine has a strong absorption band at 330 nm, and is the only base (in this tRNA) known to have an absorption band in the 300–350-nm region. Even when 4-thiouridine forms a cross linkage with cytidine, it shows an absorption band of nearly equal intensity at 330 nm (ref. 8).

Third, none of the Raman lines in question has the Raman frequencies (1,621, 1,482, 1,243, 1,160, and 708 cm^{-1}) of 4-thiouridine itself (see Fig. 2*b*). The 708 cm^{-1} line of 4-thiouridine can be assigned to the C=S stretching vibration. In the Raman spectrum now in question, on the other hand, no line assignable to the C=S stretching vibration is found. Thus, a structure lacking a C=S bond is suggested.

Fourth, *E. coli* tyrosine tRNA(2), which has 4-thiouridine residues (see, for example, ref. 9), (two at positions 8 and 9), but no cytidine residue[10] at position 13 shows the Raman lines of 4-thiouridine. The sample is fluorescent, but on the fluorescence background all of the five stronger Raman lines of 4-thiouridine can be identified (Fig. 2*a*).

Fifth, *E. coli* glutamic acid tRNA(2), which has no 4-thiouridine residue[11], has been found to give no Raman lines in these conditions. Sixth, when a bulk tRNA sample of *E. coli* is placed in a rotating cell[12] the Raman lines of 4-thiouridine do appear, but after a few runs of the spectrum with the ultraviolet laser they are gradually replaced by the Raman lines discussed.

The photochemical modification (probably the S^4U–C linking) is considered to be caused in this case by the laser beam itself. When the rotating cell is used, the chance of the laser photon hitting the intact tRNA molecule would increase, so that the Raman lines of 4-thiouridine free from the cross linkage appear.

Most of the *E. coli* tRNAs have one or two 4-thiouridine residues. Using a similar Raman spectroscopic examination we found that tRNA^{Gly} should have the 4-thiouridine residue, and that this must be involved in a similar photochemical modification (probably the 4-thiouridine-cytidine cross linkage) (see Fig. 1*b*). (Although no 4-thiouridine has been reported[13] in the primary sequences of tRNA^{Gly}, chemical analysis have shown[14] that this tRNA contains 1 mol of 4-thiouridine.) Also for tRNA^{fMet} (ref. 15) of *Thermus thermophilus*, an extremely thermophilic bacterium[16], a similar Raman spectrum attributable to the 4-thiouridine-cytidine cross linkage has been observed.

The ultraviolet laser would also be useful in obtaining information for other chromophore groups in tRNAs, for example, the Y base in yeast tRNA^{Phe} (ref. 17).

We thank Professor H. Hayatsu, University of Tokyo, for providing the sample of 4-thiouridine.

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Received November 20, 1975; accepted January 29, 1976.

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reviews

New Zealand archipelago

Malcolm Coe

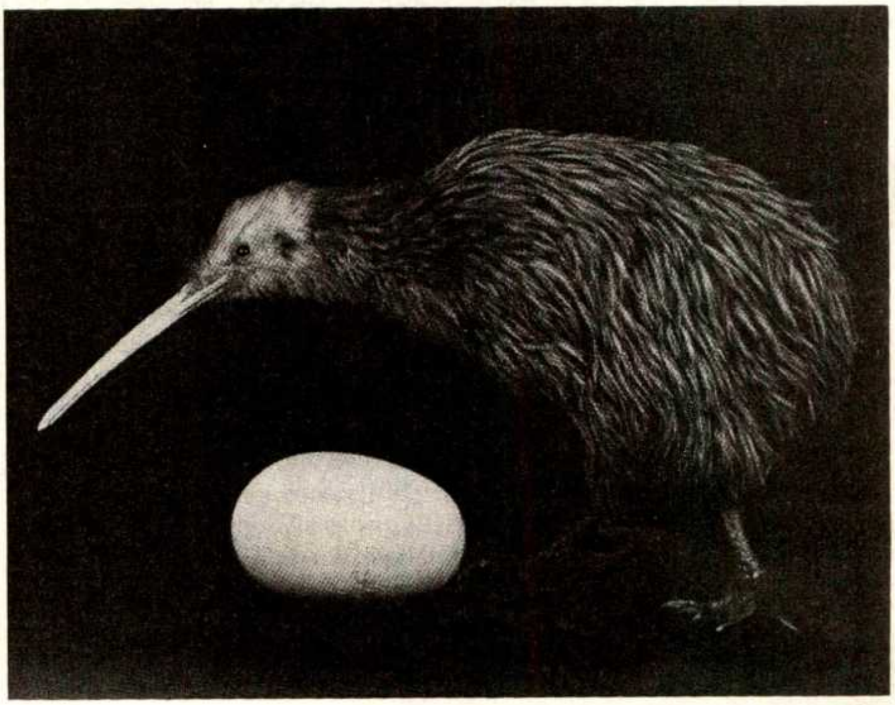


Photo: National Museum

North Island brown kiwi, Apteryx a. mantelli, *female (440 g, 125 × 78.5 mm) and egg (400 ml)*

At a time when the trend is towards publication of specialist volumes dealing with individual animal and plant groups, this broad approach* will be welcomed by the biologist who is anxious to obtain an overall assessment of an area's fauna. In using other volumes in this interesting series, I have always felt that much would have been gained by attempting to use the same format for each volume. One appreciates, however, the difficulty of persuading authors to write on anything other than their own particular specialist field. The comparative isolation of the New Zealand archipelago makes its study of particular interest to the biogeographer, for 80% of its flowering plants and 90% of its arthropods are endemic.

The first of 17 chapters sets out to describe the geological history of the archipelago as a background for the later account of the development and evolution of the main biota. In a concise account of an isolated flora and fauna, one is reminded of the drastic effect man has had on this environment when he first arrived there a 1,000 or so years ago and exterminated the moas, several species of Carinate birds and even a variety of previously prominent invertebrates. For those who still doubt the association of man with dramatic post-Pleistocene extinction it should make interesting reading. This account is followed by two chapters dealing with the climate and soils which form essential background reading on the description of vegetation which follows.

The story of man-induced changes in the biota is a continuing theme throughout the volume and considering how comparatively recent the changes are it is the more remarkable when we consider the influence that such features as the extermination of moas must have had on a vegetation for which they were the dominant large herbivore.

Although the terrestrial vertebrate fauna is only represented by 100 species, comprising 30 reptiles, 65 land birds and two mammals, this small number represents a fascinating illustration of adaptive radiation in isolation, of which probably the development of the terrestrial parrots is perhaps the most dramatic. Considering that a further 70 species of exotic birds and mammals have been introduced to New Zealand, it is surprising that even more elements of the endemic fauna have not become extinct. The fishes, in contrast to other vertebrate groups, comprise 8 families, none of which are endemic; and perhaps the greatest feature of interest is their ability to move between fresh and salt water, indicating no doubt the route by which freshwater habitats in New Zealand have been occupied. The kiwi and *Sphenodon* are justifiably given chapters of their own.

Aquatic environments are discussed in two chapters dealing with the marine benthic and freshwater biota, respectively. The greater degree of isolation experienced by the freshwater flora and fauna make them of somewhat greater biogeographical interest. Perhaps one of the greatest features of interest that emerges when we examine these 'early' groups is that comparatively few of them are represented by endemic forms, illustrating the comparative geological youth of New Zealand.

The land molluscs, arachnids, and terrestrial and freshwater insects all provide valuable examples of radiation and speciation.

The final two chapters deal with the adaptation and change in Maori culture and the influence of man on the biota. I found these two chapters disappointing, for having learned a great deal about the flora and fauna of New Zealand, the great gap in our knowledge lies in considering the ecology of the early colonists in relation to the landscape change, which has perhaps been more dramatic in New Zealand than almost any other part of the Earth's surface.

It is easy to criticise the features either included in or excluded from this volume but the resulting work will be an important source book for all students of the biogeography of Australasia. □

**Biogeography and Ecology in New Zealand.* (Monographiae Biological, Volume 27). Edited by G. Kuschel. Pp. xvi + 689. (Junk, Hague, 1975.) 200 Dutch guilders.

Plasma proteins

The Plasma Proteins: Structure, Function and Genetic Control, Volume 1. Edited by Frank W. Putnam. Second Edition. Pp. xvi+481. (Academic: New York and London, October 1975.) $37.50; £19.50.

THE appearance of volume 1 of the second edition of *The Plasma Proteins* is to be welcomed for several reasons. Most important is that it provides a survey of the multifarious developments which have occurred in the 15 years following the publication of the first edition. The mass of data now available finally decided the editor to subdivide the authorship so that a number of specialists are now responsible for particular chapters. As is stated in the introduction, however, this approach was adopted with some hesitation because of the danger that an integrated coverage might be lost. An initial attempt by the editor to write the whole of the second edition himself became impossible because "the rate of progress in plasma proteins outstripped my pen". Finally, in volume 1 only two chapters, constituting a general survey of the plasma proteins and a chapter on transferrin, have been written by the editor. The survey is valuable but, in view of the high price of the volume, a case can be made out that a wider readership would have been reached if this survey were to have appeared separately.

The second reason for welcoming this book is that it is well up to date. Probably because of the desirability for rapid publication, a few minor matters were not tidied up—for example, on p279 appears a warning which was already given on p272 that the iron-saturated and iron-free forms of transferrin can only be separated on starch gel if the starch is iron free. Also both Glx and Glu appear as abbreviations for glutamic acid. Table 1 of Chapter 2 represents a useful updating of the previous list of properties of plasma proteins. It is unfortunate, however, that reference to the original papers from which the data has been taken is indirect by way of another chapter in volumes 1, 2 or 3.

Since the appearance of the first edition, patient work in many laboratories has brought to light the complete sequences of a number of plasma proteins. Thus it may be appropriate to enquire as to the return which has resulted so far from this immense investment of time and energy. A positive answer in respect of the H and L immunoglobulin chains, albumin and the α_1 acid glycoprotein may be given with confidence. As is explained in chapter 3, the division of the albumin molecule into domains and the structures associated with the binding sites are now known. For α_1 acid glycoprotein the outcome is even more satisfactory since it is now apparent that this protein is the first single-chain plasma protein having some homology with two other plasma proteins: IgG and haptoglobin. These results suggest that the time points at which each of the currently existing plasma proteins branched off from their primitive precursors will become known.

A. H. Gordon

On-line computing

Laboratory On-Line Computing: An Introduction for Engineers and Physicists. By J. E. Brignell and G. M. Rhodes. Pp. x+297. (International Textbooks: Leighton Buzzard, September 1975.) £8.75.

THIS book sets out to introduce engineers and physicists to on-line computing. The authors wisely confine themselves mostly to generalities but at the same time there is sufficient depth of detail to make this book of real use to persons entering this complex and rapidly developing field.

Two early chapters introduce the reader to the basic elements of both hardware and software, assuming a level of knowledge usually gained in most courses in engineering or physics—a good starting point. But it is in getting to grips with the real problem—hardware–software interaction—that the authors excel, with their ability to delineate and explain the essential features of interfacing. Many complex design features of both hardware and software are explained with a lucidity which is both refreshing and instructive.

The authors devote a large section of their book to a much neglected subject—the consequences of interfacing the real continuous world to the discretised world of the digital computer. The reader is introduced very gently to theories of sampling and digital filtering.

There are, however, one or two surprising omissions. There is an absence of detailed comment on, for example, either the influence of operating systems on real-time response, or on modern programming techniques which are important for memory-starved minicomputers, such as subroutine re-entrancy. On the hardware side the ubiquitous asynchronous serial line unit is not mentioned.

On the whole, however, this is an excellent book and anyone planning to "go on-line" will certainly find it of great benefit. Research workers and engineers already experienced in on-line computing will find sections which make interesting and valuable reading.

P. F. Kilty

Organometallics

Organometallic Derivatives of the Main Group Elements. (International Review of Science. Inorganic Chemistry Series Two, Vol. 4.) Edited by B. J. Aylett. Pp. 417. (Butterworth: London; University Park Press: Baltimore, Maryland, August 1975.) £13.45.

ORGANOMETALLIC chemists are well served by surveys intended to ease the task of keeping abreast of the flood of papers on the subject. The Annual Surveys, forming part of the *Journal of Organometallic Chemistry,* provide detailed coverage of derivatives of individual metals or groups of metals; the Specialist Periodical Reports of The Chemical Society cover the whole field annually; and the series of which this volume forms a part covers derivatives of the main group elements, including group IIB, biennially (organic derivatives of the transition metals are included in other volumes in the Inorganic Chemistry series).

In this volume, chapters devoted to individual metals or related groups of metals review papers published in 1971 and 1972 (for arsenic, antimony and bismuth, early 1971 to March 1973; and for beryllium, magnesium and the alkaline earths, 1969 to 1972). The surveys are inevitably selective, and the judgment of the authors in their choice of significant papers can only be tested by subsequent developments. One omission did seem surprising: although work on various metallocarboranes is reported, there is almost no coverage of carboranes *per se.*

If the unavoidable limitations of this type of survey are accepted, this volume provides a useful key to the original literature, and the price is not excessive. There are, however, some defects, which it is hoped the publishers and editors will remedy in future editions: the apparently random order of the chapters, and the inconsistency in their titles; the brevity of the subject index (and absence of an author index); and the fact that a survey of the literature for 1971–72 has not appeared until August 1975.

B. J. Wakefield

Polychloroaromatics

Polychloroaromatic Compounds. Edited by H. Suschitzky. Pp. viii+543. (Plenum: New York and London, 1975.) $42.

MOST readers will find this book of greatest interest if they read first the last chapter, which is a comprehensive and stimulating survey of polychloroaromatic and -heteroaromatic compounds of industrial importance, by M. B. Green of ICI Mond Division. Insecticides, fungicides, herbicides and others are covered together with certain polymers and dyestuffs. This material gives special point to the earlier chapters.

In the first of these, Ballester and Olivella provide a lengthy account of aromatic and alkaromatic chlorocarbons which is clear, comprehensive and well organised. It contains very interesting material on relevant radicals, reactive and inert, and on carbonium ions and carbanions. It is followed by similar chapters on polychloroheteroaromatic compounds and on polychloroaryl derivatives of metals and metalloids by Iddon and Suschitzky and by Chivers and Wakefield, respectively.

The range of heterocyclic compounds covered is wide, including mainly mono- and bi-cyclic compounds containing one or two heteroatoms (nitrogen, oxygen and sulphur). Similarly, the coverage of metals and metalloids is comprehensive with major emphasis on alkali metals, group II metals, and group IIIA and IVA elements, particularly silicon.

The intrinsic chemical interest attached to all these compounds lies in an attempt to understand their unusual properties in terms of the cumulative electronic effects of the chlorine substituents and corresponding steric effects. Some progress in this direction has been made. The book has a traditional 'preparation and properties' flavour, but is a mine of information, indicative of many problems needing further research.

The book is well conceived and well produced, and each chapter has a full set of references. There is also a satisfactory author and subject index. It is, however, somewhat specialised for most organic chemists. Nevertheless they are all in Professor Suschitzky's debt for making available so conveniently a vast amount of useful and interesting information.

N. B. Chapman

Idealised models

Branching Processes with Biological Applications. (Wiley Series in Probability and Mathematical Statistics.) By P. Jagers. Pp. xiii+268. (Wiley Interscience: London and New York, October 1975.) £10.50.

ALTHOUGH biological populations often develop in extremely complex patterns, we may imagine idealised situations in which the underlying reproductive process has a simple structure which is free from the usual dependencies of competition, predation and the limitation of natural resources. Such simplified models lend themselves to a mathematical analysis rich in results, and the conclusions drawn from them often give great insight into the behavioural structure of the more complicated processes to which they relate. This mathematical theory has been studied in great generality and the results may be easily interpreted in a practical context.

The basic structure of such idealised models involves the concept of a branching process, in which a population of identical individuals develops in time as the individuals multiply independently of each other and eventually die. As a thorough survey of problems involving multitype branching processes is already in existence (Mode, American Elsevier, New York, 1971), their existence is mainly avoided in this book.

Within this framework questions may be asked which relate to the probability that a population becomes extinct, to the existence and structure of stable age distributions, to limiting rates of growth, and so on. The general theory surrounding such questions is developed in the first seven chapters, and applications are discussed in the last two. The reader is gently led into the text by way of a historical survey and an analysis of the simplest model available, namely the Galton–Watson process. Generalisations of this special case are considered and the first part of the text concludes with a short chapter on multi-type processes. In a brief interlude the reader is armed with the basic tools of martingales, renewal theory and point processes; and he is then presented with a full treatment of the general theory with all its attendant extensions and ramifications. These complex ideas are then illustrated by reference to several stimulating problems in the fields of demography and cell kinetics.

Dr Jagers writes in an extremely interesting literary style, rare for a mathematician, and his book is a delight to read. A high level of mathematical ability is required for full comprehension of all the theory contained in it, but it is to be hoped that biologists with little mathematical expertise will still find that his conclusions arouse considerable food for thought

E. Renshaw

Proteins

The Proteins. Volume 1. By Hans Neurath, Robert L. Hill and Carol-Leigh Boeder. Third Edition. Pp. xiii+547. (Academic: New York and London, 1975.) n.p.

THE two previous editions of *The Proteins* have achieved an honoured place in a literature that is fast attaining a complexity matched only by its subject. Is a new edition able to escape the ridicule that in these days such an all-embracing title immediately inspires? Only by the editors quickly informing the reader that generalities and unifying principles will be stressed. Eight volumes are envisaged, of which the first three will deal with general methods, and the remaining five with specific proteins that have unusual structural features or unique biological functions.

This first volume begins well with an admirable account of gel-chromatographic methods of analysis by G. K. Ackers. In view of the extent to which these methods have established themselves in protein chemistry, this seems a proper choice. In the next chapter J. Porath and T. Kristiansen describe the even more significant technique of affinity chromatography, which has achieved a major breakthrough in the last two or three years. The third chapter treats the electrophoresis of proteins in dissociated form in the presence of sodium dodecyl sulphate. A critical account is given by two of the most experienced workers in this field, K. Weber and M. Osborn. In a fourth chapter, K. E. van Holde considers sedimentation analysis. This is a topic where data analysis, and on-line computerisation, is proceeding apace behind the scenes, and we can regard this as a holding exercise for future developments. In chapter five, I. M. Klotz, D. W. Darnall and N. R. Langerman summarise what is known about the quaternary structure of some 300 proteins and describe new methods for chemically cross-linking subunits to detect near neighbours. They also bring the reader into contact with the flourishing study of protein hybrids.

The final chapter on electron microscopy by J. T. Finch provides a very thorough review of the application of image analysis to the determination of the quaternary structure of assemblies of protein molecules.

This is a very good book, written by experts who have had plenty of practice with earlier articles elsewhere.

G. A. Gilbert

Advances in Molten Salt Chemistry, Volume 3. Edited by J. Braunstein, Gleb Mamantov and G. P. Smith. Pp. xi+458. (Plenum: New York and London, 1975.) $46.20.

"MOLECULAR dynamics calculations of molten ionic salts", by L. V. Woodcock (chapter 1), is a comprehensive review of the methods and application of computer simulation techniques to interpreting the physical chemistry of molten salts. The author concludes that computer simulation offers the only presently viable approach to quantitative interpretation of ionic melt properties. "Gas solubility in molten salts" by P. E. Field reviews the literature up to 1972 and includes results for 14 gases in pure and mixed metal salts of seven anions. "Organic reactions in molten tetrachloroaluminate solvents" by H. L. Jones and R. A. Osteryoung concentrates on condensation-addition reactions, dehydrogenation-addition reactions and rearrangement-isomerisation reactions. The continuing importance of fluorides justifies a major review of "Experimental techniques in molten fluoride chemistry" by C. E. Bamberger. The detailed explanations will be useful to even a beginner in the field.

"The chemistry of thiocyanate melts" by D. H. Kerridge is the first review of the subject, covering thiocyanate melts in most groups of elements. The final chapter (171 pages) by R. E. Thoma describes "Phase diagrams of binary and ternary fluoride systems". This exhaustively catalogues every known phase diagram in which the liquidus is delineated. Hitherto unpublished data from Oak Ridge Laboratory are included, and the publication in English of much Russian literature is invaluable.

This book maintains the high standards of literary and technical excellence which have characterised the series. **H. C. Brookes**

Halonium Ions. (Reactive Intermediates in Organic Chemistry.) By George A. Olah. Pp. xiii+190. (Wiley-Interscience: New York and London, November 1975.) $22.75; £11.40.

THIS new book from Professor Olah is about species of the type $R\overset{+}{X}R'$, where R and R′ may be alkyl or aryl (including cyclic forms), and X a halogen. Aromatic iodonium salts ($Ar\overset{+}{I}Ar\ X^-$) were discovered in 1894 and are described in many standard organic texts. Aliphatic halonium ions are more recent and much of the work on them has emerged from Olah's laboratory in the last decade.

The book describes the preparations, spectroscopy (mainly nuclear magnetic resonance (NMR)) and chemical reactions (alkylation, arylation, nucleophilic attack) of the various types of halonium ion. Stability falls as the groups R change from aromatic to aliphatic and the halogen from iodine to chlorine (fluoronium ions are unknown). The wholly aliphatic $R\overset{+}{X}R'$ ions exist only at low temperatures and there is only NMR evidence (plausible rather than rigorous) for them. Olah is perhaps too certain here; although the balance of probability lies in favour of the halonium ions, more evidence that some of them are not present mainly as carbenium ions is surely needed. **J. Burdon**

Books brief

Statistical Prediction Analysis. By J. Aitchison and I. R. Dunsmore. Pp. xi+273. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £8.50.

ONE of the most important problems in applied statistics is that of predicting, on the basis of a number of observations, what a future observation will look like. In its simplest form, $x_1, x_2, \ldots x_n$ are n realisations of a random variable—what can be said about x_{n+1}? Although study of the problem goes back to Laplace and his law of succession, it does not occupy a prominent position in modern statistical thinking. This is a pity in view of the importance and frequent occurrence of the problem. The present book is a serious and successful attempt to remedy this situation. The introductory chapter contains some excellent examples and later chapters deal with applications to sampling inspection, regulation, optimisation, calibration, diagnosis and treatment allocation. Throughout the book, and particularly in these chapters, the theory is intimately related to practice and non-trivial examples are given in commendable detail. The authors have had to make an agonising decision about the mathematical level of the book. What they have chosen to do is to throw the reader in at the deep end after the gentle introductory chapter. He is sharply immersed in a whole series of distributions, and without some experience of probability distributions he will certainly be lost. The authors ease his way by full and intelligent tabulation of the distributions and by very clear writing. Within the limits of size they could hardly have done better. The philosophy of the book is largely Bayesian, although there are some deviations in chapters 5 and 6; but I think the reader will be so convinced by the good, sound common sense and practical relevance of the examples that he will scarcely realise that a serious point is at stake. This book is an important contribution to the statistical literature. **D. V. Lindley**

Chemistry, Biology, and Clinical Uses of Nucleoside Analogs. (Annals of the New York Academy of Sciences, Volume 255). Edited by A. Bloch. Pp. 610. (New York Academy of Sciences: New York, August 1975.) $46.00.

NUCLEOSIDE analogues have been used with increasing success over the past 20 years in the treatment of cancer and of bacterial viral and parasitic infections. Add to this the clinically useful immunosuppressive properties of some analogues and it is clear that they represent a therapeutically important group of compounds. In addition, some nucleoside analogues have other, perhaps no less important, actions as cardiovascular agents, specific fungicides, radioprotective agents, insect chemosterilants and as regulators of cell function. Some analogues are even growth stimulators (cytokinins), and on a socially responsible note, could, "in the food-short world of tomorrow, conceivably take on great importance". Such is the diversity of the biological properties of the agents described in this volume, that it is now evident that new analogues once isolated or synthesised, should be widely screened, since they may have any one of a number of important properties. Tubercidin (7-deaza-adenine), for instance, was rejected as a clinically useful anti-tubercular or antitumour agent because of unacceptable host toxicity. Since no toxicity is encountered on local administration to the skin, however, the agent now has potential value in the treatment of basal cell carcinoma.

This elegantly edited volume is a clear, interesting, and in places fascinating, account of nucleoside analogues, their present day uses and their future. Details are given of new synthetic routes, of the properties of novel analogues and of new ways of using already well-established agents. As the final chapter emphasises, there is still a great deal to be learnt from the study of nucleoside analogues. **T. A. Connors**

obituary

Theodosius Grigorievich Dobzhansky, world renowned population geneticist and evolutionist, died on December 18, 1975 in Davis, California. Correspondence concerning journal articles received by his colleagues suggest that he was working until the moment of death.

Dobzhansky was born on January 25, 1900 at Nemirov, near Kiev, in the USSR, where he studied biology at the University. After graduating, he became a Lecturer in Genetics at the University of Leningrad. In 1927 he received a Rockefeller Foundation fellowship to study with T. H. Morgan at Columbia University and at the California Institute of Technology, when Morgan moved there. Dobzhansky joined the faculty of the Institute after the expiry of his fellowship. He remained there as Professor of Genetics until he returned to Columbia University in 1940. In 1962 Dobzhansky joined the research staff of the Rockefeller Institute (later Rockefeller University) in New York City. On his retirement in 1971, he moved to the University of California at Davis, where he worked until his death.

Early in his career, Dobzhansky ('Dodik' or, as his American friends came to call him, 'Doby') led an expedition into central Asia studying the genetics of domestic animals. He developed a lifelong passion for horseback riding during that trip. Subsequently, nearly every site for field studies was selected to include accessibility to a riding stable; a ride of several hours on a 'splendid animal' was an essential feature of his daily routine.

Until joining Morgan's group, Dobzhansky spent some ten years studying the taxonomy, geographic variation, and polymorphism of coccinellid (ladybird) beetles. These studies, influenced by his former professor, S. S. Chetverikov, laid the foundation upon which he built his later theories of the origin of geographic races.

After joining Morgan's group, Dobzhansky started his work on Drosophila. He commenced a number of studies that would now be regarded as 'classical' genetics: in respect to the question of the pleiotropic (multiple) action of genes, he showed that a number of common mutant alleles in *D. melanogaster* had consistent effects on internal organs as well. His work on the location of gene loci through the use of chromosomal translocations led to the first cytological maps of gene loci. He pursued the genetics of sex-determination and intersexuality in Drosophila, and made valuable contributions to the then-baffling phenomenon of 'position effect'.

Dobzhansky's work on the population and evolutionary genetics of *D. pseudo-obscura* began in the early 1930s with studies on the hybrid sterility of 'inter-racial' crosses. After discovering a wealth of chromosomal inversions in these flies, he recognised that the order in which these inversions occurred could be deduced from present day gene arrangements. A phylogeny of chromosomal rearrangements spanning three species was eventually constructed.

The studies on the genetics of natural populations of *D. pseudo-obscura* and other Drosophila species were reported in a series of 43 papers, the last of which appears in the March 1976 issue of *Genetics*. These studies also formed the basis of Dobzhansky's classic text, *'Genetics and the Origin of Species'* (retitled *'Genetics of the Evolutionary Process'* in its 1970 edition). He constantly related knowledge gained firsthand from his beloved flies and beetles to appropriate biological aspects of human beings; out of such efforts came *'Mankind Evolving'*, a text that is often favourably compared with his earlier classic.

Dobzhansky will influence the course of population genetics for years to come. In addition to the 34 or so doctoral students whom he supervised, his laboratory was host to a phalanx of postdoctoral fellows from all parts of the world. At last count, Dobzhansky had been the recipient of 21 honorary degrees.

In concluding this review of Dobzhansky's many accomplishments, it is necessary to mention the vigour with which he opposed those whom he perceived as charlatans. For many years, he attacked Lysenko and Lysenkoism as that man and his followers sought to destroy both genetics and the geneticists within Stalin's USSR. Similarly, he criticised outspokenly the pseudo-science of those in the United States and elsewhere who preached the superiority of one racial group over another. And he had no patience with those who, on religious grounds, oppose evolution and the teaching of evolution in the public schools. Weakened by age and terminal disease, Dobzhansky could nevertheless muster the strength needed to thunder at a carping anti-evolutionist, "SIR! I do not question your honesty! I question your INTELLIGENCE!"

Lee Ehrman
Bruce Wallace

Professor Sir Alexander Haddow died on January 21, three days after his 69th birthday. Honours, high academic distinction and recognition of his work came to him from many institutions and countries, and these and the list of his numerous offices can be found in reference books. This is a more personal tribute and an account of his 40 years devoted to the Research Institute of the Royal Cancer Hospital (Free), known, after moving to new premises, as the Chester Beatty Research Institute.

In 1935 he arrived there from Edinburgh to join Kennaway, and in 1946 succeeded Kennaway as Director. The run-down inevitably associated with the war was reversed and he initiated a ten-year period of growth and expansion. He insisted that cancer research must not to be regarded in any narow light but must be pursued as with basic science, to which it, in turn, had to contribute. He thought himself "an anti-planner and an anti-organisation man" and he would have been appalled to hear himself described as a good administrator, yet he was just that. He provided an organisation which facilitated research, yet one that was quite free from bureaucratic complexity and he saw to it that no-one was diverted from what he described as 'their proper labour' by administrative tasks.

To achieve this took much time and effort but yet it did not stop Alex Haddow from pursuing his own research work, and from guiding the work of a large team. His knowledge of all aspects of cancer research was impressive and he took immense pains to help and advise. He never forced his own views, yet a large part of the work of the Institute, past and present, can be traced both to his guidance, enthusiasm and, not infrequently, to his own research discoveries. His attitude to research was that it was an intensely individual pursuit and that workers must be left to develop their own promise: the discipline was personal and that imposed by the subject. He impressed on all that while work had to be carefully planned, it must not be rigid to exclude or overlook the unanticipated chance or the unintended discovery which he considered to be one of the principal objectives of cancer research.

He gave up the directorship in 1969 because of his failing eyesight and retired as Professor of Experimental Pathology three years later. He did not allow this affliction to stop his work and he was active until a few weeks before his death.

His own research work, which covered a vast spectrum from the first properly conducted clinical trial of a cancer chemotherapeutic agent to studies of drug action at the molecular level, was dominated by the concept that there is a link between impairment of growth and the eventual induction of cancer. Early in his career he found that chemicals which induce cancer are almost invariably toxic to dividing cells, both normal and malignant. The full implications of this observation for the mechanisms of carcinogenesis have yet to be revealed, but for the development of the modern era of cancer chemotheraphy this finding was crucial. The study of the mode of action of carcinogens and of anticancer agents was seen by him to be closely associated, and led to the discovery within the Institute of some of the most valuable and widely used cancer chemotherapeutic agents.

While Haddow foresaw long before most the clinical possibilities of cancer chemotherapy, he appreciated the limitations of the available compounds and insisted that research efforts must be concentrated on their mode of action in the hope of achieving greater specificity. His belief that, in spite of the great diversity of carcinogens, there must be an underlying unity of mechanism and that a truly comprehensive synthesis is possible has been fully validated by recent findings that carcinogens of the widest heterogenicity induce, by various routes, very similar biochemical lesions.

His personality was complex but the key to it was his intense concern for people and causes. His idealism extended into many fields and he found the time to give active support to bodies involved with the prevention of nuclear war and the misuse of science in all its aspects, such as Pugwash. He wrote cogently on all these topics and to foster them accepted a seat on the Press Council and the advisory council of the B.B.C. He also contributed greatly to the international organisation of cancer research, both through the International Union, in which he served as President, and by personal contact.

His concern for people determined the atmosphere of the Institute. He was tolerant, generous, had an intense interest in the well-being of all who worked for him and with him and went to endless trouble to help with professional and personal problems. Haddow's immense mental and physical energy and his versatility derived from his conviction that problems intellectual, medical or social were capable of being solved and he had no sympathy for those who skirted their direct confrontation.

Peter Alexander

announcements

International meetings

March 31–April 2. **The magnetosphere/Particle physics,** Sheffield (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX, UK).

April 2, **Chemically Active Species in Biology,** The London School of Hygiene and Tropical Medicine (Assistant Secretary, The Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

April 8–9, **Stress in Animals on Experiment,** a Symposium organised by LASA and BLAVA, Guildford (Dr P. Eaton, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 9HH).

June 7–9, **Chlorophyll-Proteins Reaction Centres and Photosynthetic Membranes,** Brookhaven (Deadline for Contributions, April 16) (Dr John M. Olson, Biology Department, Brookhaven National Laboratory, Upton, New York 11973).

June 23–25, **First International Symposium on HLA and Disease,** Paris (Deadline for Abstracts, April 28 to Professor J. Dausset, Centre Hayem, Hôpital St-Louis, 75475 Paris) (Congrès-Services, 1 rue Jules Lefèbvre, 75009 Paris, France).

July 19–22, **Fifth International Symposium on Medicinal Chemistry,** Paris (Congress secretariate: 49 rue St-Andre-des-Arts, 75006 Paris).

October 27, **Ultrasonic Spectroscopy,** London (Papers: Professor A. F. Brown, The City University, St John's Street, London EC1V 4PB; Information: The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

Person to Person

Some members of the Department of Mathematics at Edinburgh University, believe that there is scope for improved co-operation between mathematicians and R and D workers in industry, and a group has been formed to identify and work on industrial problems. They wish to hear of suitable problems and to meet representatives of the firms concerned to discuss possible co-operation.

Any problem submitted would be discussed at first by the group as a whole to decide whether the requisite skills are available. The problem would then become the main concern of one or more members, with the active support of the rest of the group for discussions and workshops.

Enquiries to The Director, Centre for Industrial Consultancy and Liaison, University of Edinburgh, 14 George Square, Edinburgh EH8 9JZ, UK.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

Other countries

Unesco. Intergovernmental Oceanographic Commission. Technical Series No. 11: Bruun Memorial Lectures. (Presented at the Eighth Session of the IOC Assembly, Unseco, Paris 5–17 November 1975.) Pp. 63. (Paris: Unesco, 1975.) [812

The Coconut Industry Board, Jamaica. 14th Report of the Research Department, July 1973/June 1974. Pp. 64. (Kingston, Jamaica: Coconut Industry Board, PO Box 204, 1975.) J$3; £1.50; US$3. [912

World Health Organization: Regional Office for Europe, Copenhagen. Report of the Regional Director, July 1974 to June 1975. Pp. 105. (Copenhagen: WHO, Regional Office for Europe, 1975.) [912

CERN—European Organisation for Nuclear Research. CERN 75–15: Terminals for the Interactive Input and Editing of Bibliographic Records on the PDP–11 Computer in the CERN Library. By A. Van Praag. Pp. 60. CERN 75–16: Effects of Nuclear Radiation on the Optical Properties of Cerium-Dopes Glass. By B. McGrath, H. Schonbacher and M. Van de Voorde. Pp. 7. CERN 75–17: Calculation and Optimization of Study Fields of Septum Dipole Magnets. By A. J. T. Holmes. Pp. 19. (Geneva: CERN, 1975.) [1012

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Chemical Physics, 1974/1975. Pp. 78. Division of Chemical Physics—Research and Facilities. Pp. 90. (Clayton, Victoria, Australia: Division of Chemical Physics, CSIRO, 1975.) [1112

Australia: CSIRO. Bulletin No. 288: Energetics of Agriculture and Food Production. By Roger M. Gifford and R. J. Millington. Pp. 29. (Melbourne: CSIRO, 1975.) [1112

Australia: CSIRO. Division of Applied Geomechanics—Research Report 1975. Pp. 90. (Mount Waverley, Victoria: Division of Applied Mechanics, CSIRO, 1975.) [1112

nature

March 18, 1976

Is the dual-support system really immutable?

Is a time of no-growth one for holding the line and stabilising institutions against erosive forces, or is it one for shaking up the system, bringing in reforms and generally ensuring that if and when growth returns a new structure is there to take advantage of it? This is a frequent question, in one of its many guises, which was asked last week by Mr Gerry Fowler, Minister of State at the Department of Education and Science when addressing heads of university chemistry departments on future government policy for scientific research. And, as if to underline the no-growth theme, later in the week the government announced that although the universities' recurrent grant for day-to-day expenditure would rise 11% to £581 million in 1976–7, this figure would be a cash limit, not to be supplemented (as in the past) for additional academic salary rises.

Mr Fowler gave the impression that his department had a fairly open mind on some of the major policy issues, or at least that views were still welcome on such matters as the customer-contractor principle, the quinquennial system and the dual-support system for university research. He also resurrected the question that many universities have been studiously avoiding for a long time: how to recognise more effectively the university teacher who is not inclined to do research as his primary occupation.

In the past year the dual-support system—the concept that one organisation gives general grants to support universities and provide a 'well-found' base for another organisation to support specific research projects —has been given some fairly widespread support. A Commons select committee has positively been angling for criticism of the system but has found relatively little; such criticism as there has been has tended to be about alleged shabby treatment by one arm or the other rather than about the concept as such. So should we all settle back and let the system run on as it is?

There seem to be two defects which have not been adequately discussed yet. The first was raised by Mr Fowler himself—the university teacher. The dual-support system should offer every encouragement to the teacher who wishes only to take advantage of the well-found base and not to go after research council money. But somehow academics have got it into their heads that the badge of office is incomplete without a research grant. Second, although the system should be a support to the teacher, it can very easily be a shelter to the mediocre whose proposals to research council are regularly turned down. There has recently been much talk, not all of it malevolent, about eliminating mediocre people, departments or even universities. At present the machinery for so doing would be cumbersome in the extreme. One prop can be knocked out, but hardly both simultaneously. Would any modification of the present system of financial support provide a more challenging environment without making the rôle of the teacher even more insecure?

Don't despise the miracle

Mr John Fagan of Glasgow, suffering from a stomach cancer, was on the verge of death. The doctors had given him up, and fully expected him to die within a day or two. But his fellow-parishioners from the Roman Catholic church of The Blessed John Ogilvie were busy praying to John Ogilvie, who had been executed in 1615 for refusing to recognise the supremacy of King James I on spiritual matters. Mr Fagan survived. Nobody could find any physical cause for the dramatic turn-around in his health, and now the Roman Catholic Church has accepted the cure as miraculous and set John Ogilvie on the way to becoming a saint. At which you might expect us to sneer.

After all, there are plenty of cases where remission has occurred inexplicably; one-in-a-million chances do turn up once every million times. And what about all those cases, not given publicity by the church, where saints have been prayed to and have failed to deliver a cure? Surely in a world of scientific objectivity there can be no room for the miracle cure; the church is capitalising on rare and random phenomena.

This laboratory-bench view shows well the limited horizons that we may impose on ourselves. For we cannot yet cure cancer, least of all of the advanced sort that Mr Fagan was afflicted with, in spite of the hundreds of thousands of man-years devoted to scientific study of the subject; does that allow us to pass judgement on others who are also groping, but in entirely different territory? It does not.

It might if we were successful in curing cancer and if the church were actively dissuading its members from accepting medical treatment as a substitute for prayer to the saints. It might if the church were, as do many purveyors of quack cures, to claim a high success rate and to demand a large fee. Until then it were better that saints and scientists co-exist and be thankful for any small mercy, whether it be Mr Fagan's cure or a flash of insight accorded to some cancer researcher.

Picture: Mary Evans

Frozen mineral waters

Allan Piper looks at the obstacles to exploitation of the vast and varied resources of Antarctica

ASSESSMENTS of the economic potential of Antarctica involve issues stretching far beyond the obvious questions of how much and what sort of wealth might be there for the taking. With conditions on the continent hostile and often unpredictable, the successful operation of man, machines and extraction plant is hampered by extremes of climatic behaviour. Huge costs are imposed by Antarctica's extreme distances from the major centres of the commercial world. From the tip of the Antarctic Peninsula the journey to Cape Town is over 4,000 miles, and distances to the principal ports of the Northern Hemisphere exceed 7,000 miles. Moreover, during the winter months much of the continent is inaccessible by sea.

But there are other problems too, problems of particular relevance to any future sessions of the Law of the Sea Conference. The legal position in Antarctica is far from certain because nobody has been able to decide to whom the continent should belong. Territorial sovereignty is, and always has been, very much an open question, and the political jealousies run very deep. It is easy to see why. The history of Antarctica is characterised by diplomatic confusion. Conflicting claims for its discovery have never been satisfactorily settled, and in any case none of them was supported with the conventional backing of permanent occupation until over 120 years after the continent first became known around 1820.

What is certain is that a drive to satisfy commercial demands lay behind the eventual discovery of Antarctica, probably by the sealers who by the 1820s had penetrated southern waters as far as the South Shetland Islands bordering the Antarctic Peninsula; over the following century the whaling industry grew to become Antarctica's principal resource in the wake of brutal over-exploitation of the native fur seals and elephant seals. By 1959, when the Antarctic Treaty was signed, annual whale catches had risen to over 40,000.

Throughout the expansion of the whaling industry, however, commercial interest in Antarctica moved steadily back into second place as the continent became increasingly prized for its prestige value and potential strategic advantages. By the close of World War II national rivalries were running high. Seven countries—Argentina, Australia, Britain, Chile, France, Japan, and Norway—had asserted territorial claims and backed them up with permanent bases.

With 80% of Antarctica neatly divided into pie-slices the geographical borders became more satisfactorily defined than the diplomatic boundaries. Along with the seven existing claimants both the USA and the Soviet Union had established presences on the continent, but neither recognised other territorial claims and neither asserted any. Tensions between Argentina, Britain and Chile, whose claims partially overlap, had stretched to breaking point.

Taken in this context, the signing of the Antarctic Treaty in 1959 must be seen as a major diplomatic achievement. Belgium, New Zealand and South Africa joined the other nine states in agreeing to maintain Antarctica free of military presence, and to hold all territorial claims in abeyance, so that just as no new claim can be asserted, so none is recognised and none challenged.

It would be too charitable to suggest that none of the signatories had half an eye to the commercial potential of Antarctica when the treaty was signed, but nonetheless mineral exploration and exploitation rights are not mentioned—the feeling seemed to be that such a recognisably thorny issue could await the still-distant technological developments that would bring Antarctic exploitation closer to the realms of probability. Even seven years later most realistic prognoses tended towards the pessimistic: ". . . the exploitation of significant deposits", said a leading Australian expert, "presents enormous difficulties. It is very doubtful if minerals could be mined economically in the Antarctic, at least in the foreseeable future."

Plausible though they may have been at the time, such assessments reckoned without the economic downturn of the early 1970s, and when the signatories met in Oslo last June for the Eighth Consultative Meeting under the treaty, Antarctic resources and the likely effects of mineral exploration held centre-stage. The talks were predictably inconclusive, with a standstill agreed pending a full investigation of the environmental hazards; but the expression of environmental concern, though laudable, offers only the thinnest of disguises for the political rationale behind the delay. Exploration surveys, under the guise of scientific research, are well within the bounds of the treaty, but relationships stand to be irreparably undermined if exploitable resources are uncovered and raise again the intractable issue of sovereignty.

Antarctica's resources

The main resources are fossil fuels. Pre-continental drift reconstructions of the former Gondwana continent indicate that most of the Antarctic continental shelf includes thick sedimentary sequences likely to contain fuel reserves. The research vessel Glomar Challenger has already found evidence of natural gas beneath the Ross Sea, and an American estimate has put reserves in the eastern shelf at around 115 million million cubic feet of natural gas and 45 thousand million barrels of oil —almost ten times the estimated combined oil reserves of the Brent, Ninian and Forties fields of the North Sea. The chances of uncovering significant deposits have become so good that at the close of the Oslo talks one American delegate was moved to ponder openly the political effect of a rich strike beneath the zone disputed by Argentina, Britain and Chile.

Evidently the Soviet Union finds the notion equally as intriguing, for less than three weeks later it announced plans to open a new survey base on the Filchner Ice Shelf at the southern end of the Weddell Sea, an area within the disputed territory. Though in itself not a contravention of the treaty, the development is significant because the Soviet Union has never previously shown any interest whatsoever in that part of Antarctica. The Soviet targets are clearly fossil fuels and metallic ores, and it does not require an unduly cynical appraisal to conclude that the main objective of the move is to force an early definition of mineral exploitation rights. "The eastern part of the region", the official statement said, "has many common features with South Africa, the south is reminiscent of the ore-bearing zones of Siberia, while the west is a continuation of the American mountains, famous for their deposits of non-ferrous minerals."

The statement in no way overstates the possibilities, and even though prospecting techniques will be hampered by the enormous overlay of ice, the chances of small Soviet successes must be rated as reasonable. The sedimentary accumulation beneath the Weddel Sea, like those beneath the Ross and Bellingshausen seas, includes up to 15,000 feet of potentially productive strata. The once-contiguous continental shelves off South Africa and South America are already known to contain small fossil fuel reserves.

Opportunities for the discovery of coal reserves and mineral deposits are equally as good on the main landmass. The extensive coal deposits which crop out throughout eastern Antarctica, particularly in the Horlick, Prince Charles and Pensacola Mountains, and in Victoria Land and the George V Coast, suggest that reserves within Permian sandstones may extend at depth beneath the entire continent. As for metallic and non-metallic minerals, over 200 species have been recorded from localities throughout the continent over the past 75 years. Chromium, cobalt, copper, diamond, manganese, mercury, molybdenum, platinum and uranium are all present, and trace quantities of silver and gold, in amounts of up to 2 and 10 parts per million respectively, have been found in pyrite-rich rocks on the Antarctic Peninsula.

But even after the recent adjustments of world commodity prices, has the exploitation of Antarctic resources become an economic possibility? The answer for the moment must be no. Extracting fossil fuels is hazardous enough in the North Sea, where tens of lives are lost to the industry annually, and where unpredictable climatic behaviour has already pushed production costs way above the earliest estimates. Conditions in Antarctic waters are considerably worse. The depth of water overlying the Antarctic shelves is more than double the average 700 feet in the North Sea, and the insurmountable obstructions of floating pack ice and icebergs scouring the sea floor mean that even such technological innovations as flexible drilling apparatus are unlikely to prove feasible.

Just as daunting and commercially restrictive are the handicaps on the continent itself. Most deposits are isolated, away from the coast, and cut off by glaciers. Coal beds are in the majority of cases limited in their horizontal extent, and mineral finds are usually reported from glacial morraines, so that original outcrops are unknown. Moreover, quality and tenor are generally poor. As for the other minerals, not even high-value metals such as silver, uranium and gold will become viable failing unlikely upward shifts in world prices. The costs of USA gold production would need approximately to double before Antarctic deposits became economic.

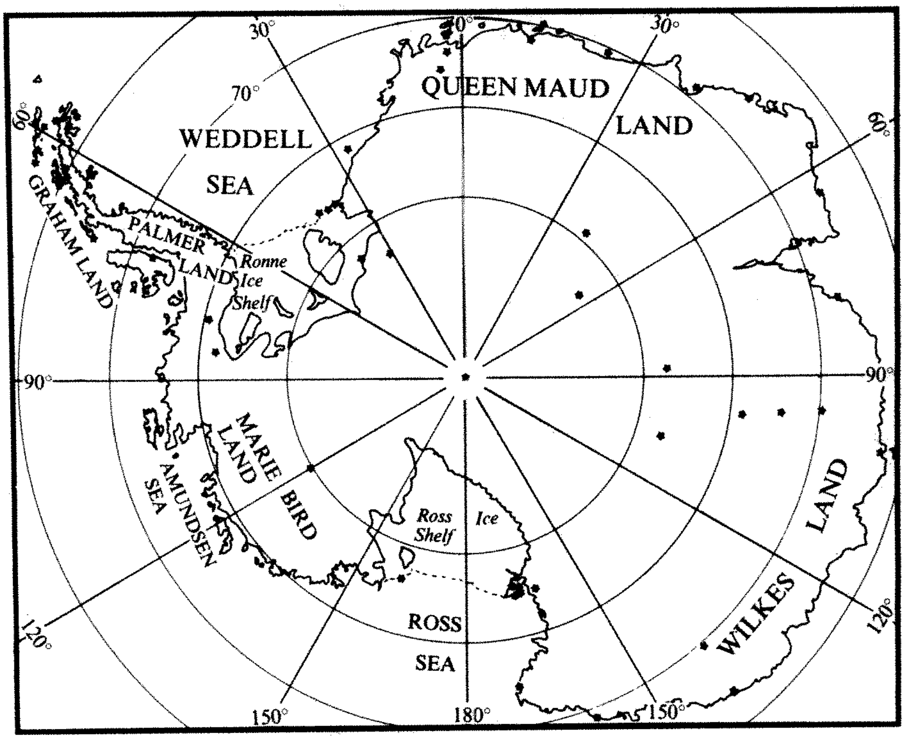
Exceptions

But any thorough assessment of the opportunities must take into account two possible exceptions to this general perspective. The first involves the manganese nodules lining the ocean floor between the continental shelf and the outer boundary of Antarctic territory at the relatively temperate latitude of 60°S. Manganese nodules are present throughout the world, and with the development of suitable extraction technology are likely to prove an extremely rich resource.

Just as promising is the economic potential of the igneous Dufek Massif in the northern Pensacola Mountains. So far, only viable deposits of iron-bearing magnetite ores have been found, but the complex so closely resembles the richly productive Bushveld intrusion of southern Africa that the prospects have to be viewed in a sanguine light. Further, there is no reason why the Dufek Massif need be unique in Antarctica.

In the final analysis, however, it is the biological resources of Antarctica which currently offer the greatest prospects for this future. Seals are now adequately protected under the Antarctic Treaty, and the whaling industry, which has declined from its level of 15 years ago, will probably continue to do so under the likely build-up of adverse world pressure. Even so, Antarctic waters are stocked with a superabundance of krill, *Euphausia superba*, the small shrimp-like creature forming the staple diet of the whales that visit the region to gorge themselves each year. One estimate has suggested that factory ships could economically take an annual minimum of 270 million tons—an amount far in excess of present yearly world fish catches—without upsetting natural balances.

As for the question of mineral exploitation, this will be discussed when the signatories to the Antarctic Treaty gather in London this summer for a preparatory session ahead of their Ninth Consultative Meeting in Paris next year. □



Antarctica, showing scientific stations in operation 1954–1975

An erosion of eminence?

Colin Norman reports from Washington on a document that examines the strengths and weaknesses in American science

AMERICAN pre-eminence in most areas of science and technology is gradually being eroded, largely because inflation has been eating into funds for research and development in the United States and many other countries have been ploughing large resources into their own science programmes for the past few years. That conclusion emerges from a detailed statistical portrait of American science from 1960 to 1974, published last month by the National Science Board, a committee of eminent scientists which directs the policies of the National Science Foundation.

A cautiously worded document which studiously avoids drawing many conclusions, it attempts to pinpoint strengths and weaknesses in American science and to analyse long-term trends. It covers a vast range of data, including funding levels for research and development, employment statistics, rates of innovation, output of scientific literature and the average number of citations per research paper.

Taken on its own, any one of those indicators probably doesn't mean very much, but together they seem to show a consistent trend—the quality and quantity of American science in relation to that of other countries appears to be headed downwards. The trend, in fact, is so consistent that early drafts of the report are said to have played an important role in securing a large increase for science and technology in the budget announced by President Ford last January. That budget would increase support for research and development by about 11%, while total federal expenditures would only grow by about 5.5%.

Shrinking funds

The clearest and most striking trend brought out in the report is that government funds for research and development have been shrinking steadily in terms of purchasing power since 1968. Support for basic research dwindled by 13% between 1968 and 1974, while expenditures per scientist dropped by a staggering 30% over the same period.

In an attempt to analyse trends in the United States in relation to those in other countries, the report brings out the fact that only two countries—the United States and France—have shown a long-term decline in the proportion of gross national product spent on science and technology, and the United States is alone in showing a steady decline in the number of scientists and engineers employed in research and development.

Though there have been some significant changes in the pattern of government support for research and development, the report shows that the United States devotes a larger share of its research budget to defence (about 50%) than other countries do. Over the past seven years, however, the share devoted to space science and technology has dwindled, while that devoted to other areas of science has increased.

It is difficult to measure the effect of shrinking support on the overall quality and productivity of American science, but several indicators suggest that the cuts are beginning to show some impact. One measure suggests that the proportion of research papers in several fields of science produced by Americans has reached a plateau; in some cases it has declined. The fields chosen are chemistry, physics, engineering, psychology, molecular biology, systematic biology, and mathematics; in all except chemistry and mathematics, the US still produces the largest share of the world's literature, however.

UK ranks high

As for the quality of research, the report indicates that in every field, research papers produced in the United States are cited on average more often than those produced elsewhere. According to that criterion, papers produced in the United Kingdom rank second in the fields of clinical medicine, biology, and earth and space science, while the UK ranks third in chemistry, physics and engineering. The report does not attempt to analyse whether there are any long-term changes in ranking.

As for technological invention and innovation, some indicators again suggest that American pre-eminence is being eroded by gains in other countries. One measure, the so-called 'patent balance', which relates the number of patents granted to nationals with those granted to foreigners in each country, has been dropping steadily in the United States since 1966. Over the period 1966 to 1973, the number of foreign patents granted in the United states increased by 65%, reaching 30% of the total granted in 1973. That, the report notes, "suggests that the number of patentable ideas of international merit has been growing at a greater rate in other countries than the United States".

Another striking example of that trend shows up in an analysis of rates of innovation. According to a study of some 500 innovations in various areas of science and technology, the US was producing about 80% of the world's innovations in the mid 1950s. By the mid-1960s, the US share had shrunk to about 50%, and although there has been a slight increase since, it has been caused more by a decline in the UK's share than by an improvement in the US position. (The share of technological innovations produced in Britain declined from 25% in 1965 to about 15% in 1973.)

The only indicator which seems to buck the trend of declining US leadership in science and technology is a measure of the balance of trade in high technology products. From 1960 to 1970, the US balance of payments in such products climbed steadily from about $6,000 million to about $12,000 million, but between 1972 and 1974, it suddenly jumped to $25,000 million a year.

Basic research affected

The area most severely affected by declining support in the United States is basic research. In terms of constant dollars, federal support for such research shrunk by some 13% between 1968 and 1974. Physical sciences were the hardest hit, declining by 25%, chiefly because of reductions in the space programme. Those declines have had considerable impact in the universities.

The report notes, for example, that the universities went through a period of rapid expansion until the early 1970s, with faculty numbers in science departments increasing by more than 60% between 1965 and 1972. But, because of declining funds for research, the academic job market has become relatively depressed recently. The result is that the proportion of faculty with tenure has increased from 47 to 65%, and the proportion of young doctoral faculty in postgraduate science and engineering departments declined from 42% in 1968 to 28% in 1974. The median age of faculty members consequently increased from 41 to 44.

Many of the trends and conclusions brought out in the report are hedged with caveats and cautions. The National Science Board hopes to refine some of the measures, and produce an updated report in a couple of years time, however. ☐

BRITAIN

Changing nuclear fortunes

News last week transformed uncertainty surrounding the reprocessing of nuclear fuel in Britain into certainty. For the Dragon reactor, the reverse seemed to occur. Allan Piper and Roger Woodham report

AFTER several months' delay British Nuclear Fuels Ltd (BNFL) has obtained Government approval for plans to import and reprocess 6,000 tonnes of foreign nuclear fuel. The go-ahead clears the way for an early settlement of the contract for BNFL to handle up to 4,000 tonnes of material from Japan's new generation of light water reactors at its Windscale plant in Cumbria. But further delays are expected following French moves to capture part of the deal, which is worth as much as £600 millions over the decade following 1979.

Mr Con Allday, BNFL's managing director, has indicated that the head of the French reprocessing organisation Compagnie Generale des Matieres Nucleaires (CGMN) may accompany him to Japan later this month. The French intervention, made possible by their involvement in a tripartite reprocessing alliance with Britain and West Germany, could mean the loss to BNFL of up to half of the Japanese contract.

Final settlement of the Japanese deal, which has aroused some controversy in the UK, has been delayed by a Government investigation into the issue. Granting Government approval last week, the Energy Secretary, Mr Anthony Wedgwood Benn, said that the Government had "given full consideration to the safety and environmental implications of accepting more work of this kind, taking account particularly of the views which have been expressed in the recent extensive public discussion of the question".

It is now clear that all waste, whether reprocessed or not, will eventually return to Japan, and that the deal does not contravene the Non-Proliferation Treaty. In further contracts clients must accept a break clause freeing BNFL if residual waste cannot be treated for safe return in a glassified form.

BNFL have already expressed confidence that successful completion of the Japanese negotiations will bring in other orders, and Iran, Italy, Spain, Sweden and West Germany have been unofficially mentioned in this connection. To meet projections for oxide reprocessing by 1990 BNFL plan to invest £900 millions in two new reprocessing plants at Windscale.

Meanwhile, the moribund Dragon High Temperature Reactor Project continues to attract attention. Whatever the politicians in Brussels may decide about a data retrieval programme to get the most out of the Dragon project, a sum of about £700,000 has already been committed by other interested parties to this end. The German nuclear research centre, KFA Jülich, has chipped in £480,000 to make sure that all the information it needs for its work on high temperature reactors is wrung out of the project, and even the European Commission and the United States Energy Research and Development Administration (which eventually declined at the end of last year to help keep the project afloat) are contributing to a brief continuation of the programme concerning the effects of helium in the primary cooling circuit on the metals used there. This work is likely to continue to the end of the year and involves carrying on existing Dragon contracts with the Norwegian research centre CIIR and the Fiat company in Turin.

Apart from the £130,000 which it is making available for this venture, KFA Jülich is also providing £350,000 towards a seemly winding-up of the work at Dragon on fuel elements, in which it has a particular interest. This will involve what remains of the Dragon staff in post-irradiation experiments on the test fuel, some of which is still in the reactor.

The German research centre does, however, stand to recoup that £350,000 if a more comprehensive plan put up by the European Commission survives the Brussels obstacle course. The commission is suggesting a data retrieval programme costing £1.16 million, of which it would pick up the tab for 90%—the assumption being that some of the smaller countries that have participated in Dragon would stump up the remainder, and that the UK Atomic Energy Authority would contribute nothing.

The indications are, however, that the polarisation of political views in the Council of Ministers is such that even this—an expenditure of some 2% of the total amount of money spent on Dragon since its inception in 1959 to see the project decently buried—is by no means certain to be acceptable. And, in addition, some of the smaller countries associated with the project have simply given up in disgust. ☐

AUSTRALIA

Probing the conservative mind

Peter Pockley reports from Sydney on the latest developments affecting Australia's scientific community three months into the new administration

SHELTERED from the blaze of publicity surrounding the activities of the Federal politicians as they jockeyed for position before the opening of the new parliament, Australian scientists have been working behind the scenes to protect their interests. During January and February, the Prime Minister, Mr Malcolm Fraser, was busy putting the axe into a number of Labor-initiated inquiries, commissions and authorities. As the Canberra grapevine identified the descending blades, all manner of interested groups hurriedly sought ways of protecting their areas of interest and influence. Some lost their heads in the scramble, partly for want of comprehending the conservative mind now in power. The leaders of the scientific community, though, were by then well versed in political techniques, and their burst of frenetic activity had considerable, possibly long-lasting, effect.

ASTEC survives—just

In the eyes of the scientific lobbyists, the principal institution to defend was the fledgling Interim Australian Science and Technology Council (ASTEC), which was under very real threat of abolition or absorption within the public service structure. To the

Chairing ASTEC review: Dr Louis Matheson surprise of some, for as Prime Minister he was largely an unknown quantity, Mr Fraser proved accessible to the principal lobbyists, and he demonstrated a close, personal interest in science matters.

In the few months of its existence under Labor, ASTEC had begun to work well, but this could only be perceived on an internal basis. It had not had time to show results in the form of well-considered reports and advice to government. The dozen members had defined the major policy targets to examine in depth, and they set about doing so through a series of "Task Forces", each comprising members of ASTEC and co-opted outside experts.

Task Forces on Energy, Health Science, Social Science, Marine Science and Public Understanding of Science were established as going concerns by the time Mr Whitlam's government was defeated at the polls in December. Further Task Forces on Industrial Research and National Science Budget were formed but had not met. Some criticism had been levelled at ASTEC for allowing itself to take on too much in the way of tactical detail. Some of this detail was no fault of the members, though, since quick advice was sought on occasions by the then Labor Minister for Science and Consumer Affairs, Mr Clyde Cameron.

A broader strategic approach was favoured by these critics, but it is likely that this would have evolved naturally anyway. The existence of the criticism, however, assisted the anti-ASTEC arguments of the Department of Science following the election. Butterflies fluttered in the bellies of those scientists who saw in ASTEC their best, and only, hope for policy advice which is independent of the large government establishments dominating the Australian science scene.

At the end of January, Mr Fraser published his list of cuts and abbreviations in the inquiry industry. Although many of the cuts were largely cosmetic, they carried the useful public relations impression of a tough hand at the helm of economic management by first curtailing public expenditure. It was nonetheless important that ASTEC did not appear on the list. For those involved, a further fortnight of anxiety followed, caused by the continuing uncertainties of learning to deal with a new political regime. Then, Mr Fraser personally announced the formation of an Advisory Group to advise him—significantly him—"on the role of a continuing Science and Technology Council, its terms of reference and all other matters concerning its operation."

Dr Louis Matheson, the part-time Chairman appointed by Labor to ASTEC with expectation of full-time status, is chairing the review, again in a part-time capacity. Three of the four other members are "heavies" of the Academy of Science; the numbers are made up by a retired industrialist.

The very existence of the review, now working actively, and the nature of its membership point to a recommendation for the continuance of ASTEC, but whether, in the context of a public service-oriented government, the Labor ideal of statutory independence can be sustained is more doubtful.

Labor disarray

The traumatic period of adjustment to the status of an Opposition is still eating into the heart of the Labor Party, and it is not surprising that they have been silent on science policy matters. They seem to have been too busy tearing each other apart (notably trying to downgrade their re-elected leader, Mr Gough Whitlam) to probe the government's uncertainties on science policy. The Labor shadow cabinet is an elected body from the caucus of members of both Houses. Mr. Whitlam was saddled with some shadow ministers whom he would rather not have seen again, such as Mr Rex Connor, whom he had sacked as Minister for Minerals and Energy when in government, and Mr Clyde Cameron, whom he had demoted to the portfolio of Science and Consumer Affairs.

Mr Whitlam's one area of choice were the "portfolios" allocated to his front bench. Mr Connor became spokesman for science. So far, the shadow has not materialised into substance. □

COMECON

THE Interkosmos programme for joint Comecon research in space has just celebrated its tenth anniversary. The intention behind the programme was to carry out research into four major fields—space physics, meteorology, communications and space biology and medicine. According to its chairman, Academician B. N. Petrov, it was envisaged as a cooperative effort by all Comecon countries, whose scientists "jointly drew up a catalogue" of the most interesting problems in space research. The Soviet Union has, of course, always been responsible for providing the rockets and launch facilities to place the Interkosmos probes in orbit, and theoretically all the member countries should provide instrumentation. In fact, the Soviet participants provide the major share; out of five experiments in the recent Interkosmos-14, three were of Soviet provenance, one came from Czechoslovakia and one from Bulgaria. This imbalance is despite the fact that some member states are competent to provide experiments—Poland, Czechoslovakia, Rumania and Hungary were represented, as well as France and the USA, in the experiments carried by the biological satellite Kosmos-782.

● Aid to Mongolia from the developed members of Comecon was an issue discussed when the Comecon Committee on Scientific and Technical Cooperation met at Budapest in January. Mongolia joined Comecon in 1962, and is now seen as a potential source of mineral wealth: teams from the Soviet Union, Hungary, the GDR, Poland and Czechoslovakia have carried out exhaustive geological surveys during recent years, making important finds of coal, copper, tin, gold, phosphorites and kaolin; prospecting is also going forward for tungsten, fluorite and zinc. Over the last three years, the Standing Commission of Comecon has been working out an integrated economic plan for the development of these resources. In addition to assisting with these surveying projects, the European members of Comecon will, during the coming Five Year Plan, assist the development of Mongolian science and technology in a number of ways, including the establishment of laboratories for genetics, radio-electronics, and the analysis of fuels and lubricants.

● Cooperation in the agricultural sciences between the USSR and the other member countries is an important aspect of Comecon policy, and a recent agreement between Hungary and the Soviet Union envisages joint research into the procurement, storage and processing of cereals and the production of mixed fodder. Agricultural experts in the two countries, says the Soviet Minister of Procurement, are working on a number of identical problems. But one particular problem of Hungarian agriculture—the sugar beet harvest—seems to have arisen directly as a result of copying Soviet models.

Hungarian agriculture has embarked on an extensive programme of "chemicisation", and, according to Budapest radio, while the 1975 campaign to raise sugar beet production increased both the area under beet and the crop-density, the sugar yield of the beet was actually lower than the pre-campaign level.

Vera Rich

IN BRIEF

EEC at sea

Member states of the EEC, which is currently preparing itself for 200-mile community fishing limits, went to the fourth session of the Law of the Sea Conference this week still facing a problem over national limits. Responding to the resistance expressed to the idea of a 12-mile limit by James Callaghan, the UK Foreign Secretary, at the Foreign Ministers meeting a fortnight ago, the EEC Agriculture Commissioner, M Pierre Lardinois, said last week that any extension of exclusive national limits beyond 12 miles would require a reversal of the Treaty of Accession, and indicated that catch quotas might offer a means of achieving the desired end. Delegates from over 150 countries are in New York for the Law of the Sea Conference, and apart from the problem of coastal states' jurisidiction, they are expected to tackle the issue of exclusive economic zones, particularly in relation to fishing and deep-sea mining.

ABRC confirms cutbacks

Figures contained in appendices to the second report (published this week) of the UK Advisory Board for the Research Councils (ABRC), which advises the Minister of Education and Science on the allocation of the science budget amongst the research councils, confirms the downward trend indicated by the recent public expenditure White Paper. The distribution of the £216 million science budget for 1976-77 shows that, while overall growth in real terms will amount to 1.7%, the Science Research Council (SRC) will experience a 1.6% decline. The SRC's share of the total will fall from 54.2% now to about 52% in 1980–81. The proportion going to "big science" will fall even faster, from 34% to 27%.

Asbestos check

The UK Health and Safety Executive is reportedly investigating compliance with and enforcement of the precautions contained in the 1969 Asbestos Regulations. The immediate cause of concern is a suspected link between mesothelioma (a malignant tumour in the stomach or chest) and exposure to asbestos.

Kissinger on nuclear trade

Speaking before a Senate committee last week, the US Secretary of State, Dr Henry Kissinger, stated his opposition to specific proposals designed to tighten control of international trade in nuclear technology, and rejected suggestions that the USA and USSR should jointly urge countries like France and West Germany not to export nuclear facilities. The proposals, Dr Kissinger said, would violate the spirit of international undertakings by the US.

Uranium resources report

A report on uranium resources produced by the OECD's Nuclear Energy Agency (NEA) and the International Atomic Energy Agency (IAEA) estimates that demand for natural uranium will increase from the present 18,000 tonnes a year to about 50,000 tonnes by 1980 and possibly 100,000 tonnes by 1985. Demand up to the next decade, the report indicates, could be met, but thereafter much will also depend on recycling and prevailing prices as well as new discoveries.

Ravished goddess

THOMAS H. JUKES

ONE place in California exceeds all the rest in scenery and fame: Yosemite Valley, a national and international shrine of acclaimed splendour. Two Congressional subcommittees have just recommended that the US Interior Department take immediate action to prevent Yosemite from becoming "a major Walt Disney-type attraction". Hopefully, the analogy bodes ill for an actual plan by Disney to destroy another mountain treasure: Mineral King in the southern Sierra Nevada, where restaurants, chair-lifts and garages have been proposed.

I have not seen the report, but I know some of the circumstances leading to it such as the egregious and repulsive proposal that a tramway be built on Glacier Point. This wretched idea was turned down, but the very suggestion should have been enough to call for immediate expulsion of its proponent, the Music Corporation of America (MCA), from the concessions now assigned to MCA in Yosemite.

The Valley has been described in the prose of John Muir and the poetic photography of Ansel Adams. I can do no more than footnote their achievements. Yosemite, the goddess of fatal beauty, in spring is the deep roar of stupendous waterfalls, profusions of azaleas and dogwood blossoms, soaring sculptures in immense granite cliffs, the fragrance of ponderosa pine trees mingled with new foliage of black oaks and green leaves of manzanita, the harsh call of dark-crested Steller jays.

A rock-climber, Yvon Chouinard, has spoken of, in summer

> "the tremendous heat, the dirt-filled cracks, . . . the filth and noise of Camp 4 (the climbers' campground), and, worst of all, the multitudes of tourists . . . but then there are times when I should rather be here than anywhere else in the world . . . It is a strange, passionate love that I feel for this Valley"

Clinging to the sun-warmed rock, high above the valley floor, a climber sees Yosemite better than anyone else. The sounds below fade to a faint murmur.

Golf courses, tennis courts, a bank, service stations and garages, barber and beauty shops, and nineteen places where liquor can be purchased, have invaded the delicate tissues of Yosemite like a festering sore. The best therapy would be debridement. All buildings could be removed by using the equipment and machinery that work so successfully for this purpose in run-down urban areas. It would, of course, defeat the basis of the restoration if visitors were kept out of the Valley, but the less agile could travel in sight-seeing buses (many do so now) and the more able-bodied could move on bicycles or on foot, thus combining the benefits of scenery and exercise. (Last October, I gave a lecture on nutrition in an auditorium in Yosemite. I should have asked the audience, in the name of John Muir to leave the building, forget the meeting, and walk with me to Vernal Falls).

These changes, which are doubtless too radical to be considered, would disrupt jobs and sensibilities. But their adoption would do nothing but good to Yosemite Valley. ●

correspondence

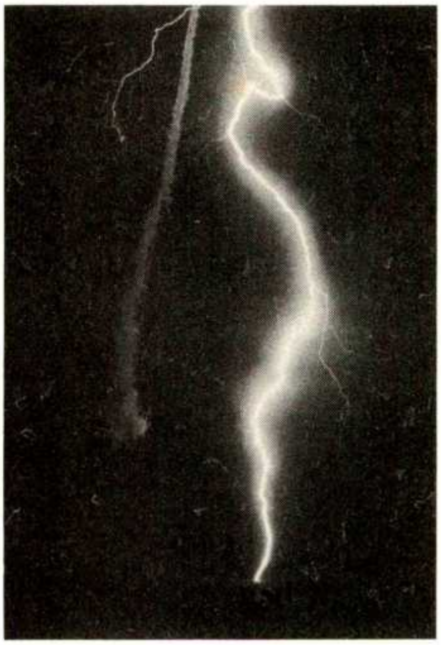
Triggered lightning hazards

SIR,—Following the publication of our paper entitled "Artificially triggered lightning above land" (*Nature*, **257**, 212; 1975) we hear that several specialists elsewhere are thinking of embarking on similar programmes. Therefore, safety considerations prompt us to discuss some recent findings about unexpected lightning strokes to the ground in the vicinity of such experiments.

Among a total of 45 successful triggerings using a rocket carrying a thin steel wire attached to the ground, the following sequence of events occurred on six occasions. First, when the ascending rocket was more than 150 m high, the device attracted a small current detected only because the vaporised wire emitted a faint glow. This glow passed through a maximum and then began to decrease. Between 40 and 80 milliseconds later, a bright flash occurred striking the ground or an object in the near neighbourhood. The maximum distance between the expected and actual point at which the lightning struck has been as much as 145 m.

Evidence obtained at various distances with still and movie cameras reveals that in the upper region the arrangement of the luminous branches is typical of upward development, whereas in the lower part the streamer shapes indicate downward propagation. Therefore it is likely that the main channel is created by propagation in both directions, starting from the upper portion of the wire.

The photograph is of an anomalously triggered flash striking the ground near our experimental site. Another flash which struck a pine grove damaged several trees and in the next twelve months these and adjacent ones died.



The anomalous triggering effect is of great interest but calls for more stringent safety measures in the experimental area. A detailed report is available on request.

Yours faithfully,

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CPSU Congress

SIR—A notable feature of the recent Twenty-Fifth Congress of the Soviet Communist Party is the adherence of the hard-core communists to Karl Marx's principle of the scientific interpretation of history.

Mr Pytor Masherov, party chief of the Byelorussian Republic, in an attack on some Western communists, said: "To say Marxism-Leninism has become antiquated is like saying that Newton's 300-year-old law of gravity is antiquated too".

Mr Gus Hall, the US Communist Party Chief, went a step further. He said: "The ideas of Marxism-Leninism penetrate the whole contents of Leonid Brezhnev's speech like the bright ray of a powerful laser".

Perhaps in the following congress we shall hear of quasars, black-holes, big bangs, etc. As a scientific community we should feel elated!

Yours faithfully,

SREEDHAN ROY

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University College,
London, UK

The pace of life

SIR,—Bornstein and Bornstein's paper (February 19, page 557) leads to fascinating conclusions. Their data suggest that in the case of the smallest human conurbation (one person) the average walking speed is 0.05 feet s^{-1} (50 m h^{-1} or 1.3 km d^{-1}). Fortunately this quantity is not negative. On the other hand, its value is small and a value of zero is probably compatible with the experimental data. This may be the explanation of the fact that those few people who are known to live alone show a propensity for spending extended periods of time on the tops of poles, in caves or in barrels.

Yours faithfully,

J. A. EADES

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Competition 6

LITTLE jingles are being put in advertisements by the British Metrication Board to speed the public's passage into a metrical world. Specimen—

Two and a quarter pounds of jam,
Weigh about a kilogram.

A prize of £10 is offered for the best rhyming couplet on one of those forgettable SI units. Closing date for entries is April 21.

Competition 5 demanded an anagram on a scientific concept to rival the irreverent one on *Nessiteras rhombopteryx*. There was a large entry—most people couldn't stop when they got into the swing of things.

To W. A. F. Watson of Aberdeen goes £6 for "Fie, Mendel! Result cheating, score *all* peas!" (These DNA molecules are self replicating).

H. J. Taylor of Sheffield wins £4 for "Natural Science men musn't interpolate inapt ideas" (Entia non sunt multiplicanda praeter necessitatem).

C. K. Thornhill of Orpington received honorable mention for "O, so in fact G not t-variant?" (Constant of gravitation). Hard luck on several good candidates whose anagrams weren't.

news and views

First mammalian results with genetic recombinants

from Bob Williamson

BREAKTHROUGHS in science occur in weird and wonderful ways. The scenarios for genetic engineering (forming recombinant molecules of bacterial virus DNA and animal or plant DNA) vary, as most now know, through the entire gamut of the fanciful—from joy to horror, depending more upon the mood of the speaker and the receptiveness of the audience than on anything as mundane as fact. During the past 2 months four reports have appeared of the first integration of portions of a mammalian gene into a bacterial plasmid—as might be expected, a portion of the gene coding for the protein haemoglobin. In the event, the recombinants obtained well illustrate the tremendous technical usefulness of genetic engineering, with little of the fiction one way or the other. For the next few years at least, recombinants will provide molecular biologists with a versatile tool to prepare pure probes with which to dissect the fine structures of genes and the way they are expressed. The experiments which will be done have been attempted before, but the probes will now be better.

Basically, all four groups have made a double-strand copy in DNA of purified globin messenger RNA prepared from rabbit reticulocytes. Since the mRNA is already very pure (as judged by direct sequence analysis, among other things) there is little chance of introducing any Pandora's box of nasties into the plasmid—the experiment must be judged one of the safest imaginable, much safer than any planned integration of DNA fractions made from mammalian cell nuclei.

In each case the first step is to make a single-strand copy of the globin mRNA with DNA-dependent RNA polymerase, the 'reverse transcriptase' isolated from tumour viruses. Rougeon, Kourilsky and Mach (*Nucleic Acids Res.*, **2**, 2365; 1975) from Geneva and Paris, prepared this in the conventional way, and then made the complementary DNA strand using DNA polymerase, giving a double-strand DNA indistinguishable from part of the globin gene. (Since cDNA is rarely a full length copy of mRNA, it is likely that the double strand globin gene DNA is only partial as well, though full data on this is not yet available.) This 'gene' was then elongated with another enzyme, terminal transferase, which adds homopolymer tails, in this case of oligo(dG), to the 3′-ends of both strands of DNA.

A plasmid, pCRI, was then joined to the partial copy of the double-strand globin gene. pCRI is derived from colEI-kan, which can be selected because of the resistance to the antibiotic kanamycin it confers on infected cells. It grows in that favourite organism of bacterial geneticists, *Escherichia coli*. The plasmid used is, like all plasmids, circular, but can be broken in just one specific place by the bacterial restriction nuclease *Eco*R1. This linear plasmid DNA was first repaired with reverse transcriptase to reconstruct the double strands at the overlapping cuts of the *Eco*R1 enzyme, and then oligo(dC) was added to both 3′-ends (again with terminal transferase).

The plasmid DNA, with its oligo(dC) tails, was mixed with the globin gene, with its oligo(dG) tails—strong hydrogen bonds form between oligo(dC) and oligo(dG). The globin gene with tails 'fills in' the rest of the nicked circle—only plasmids picking up globin genes are circular, and only a circular plasmid is infective. Therefore only recombinants which have picked up the added globin genes will grow and multiply in the bacteria. When it enters an *E. coli* cell the plasmid is held together only by hydrogen bonds, but the bacterium does the rest, and repairs the plasmid by filling in any gaps and forming covalent closed circles. The elegance of Rougeon's experimental approach is that the inserted fragment can in theory be cut out again using restriction enzymes, since each R1-sensitive site has been carefully reconstructed on either side of the inserted gene.

The results are not quite so simple—the efficiency of transformation is still rather low, and most recombinants do not contain inserted fragments—but at least one of the plasmids isolated contains a portion of the rabbit β-globin gene, as shown by hybridisation to globin cDNA (single stranded, this time) enriched in β-globin sequences. The grapevine (extraordinarily efficient in this field—I find it hard to imagine anyone getting a positive result without everyone else knowing within days at most) tells us that an α-globin gene recombinant has also been prepared by this group.

Tom Maniatis and his colleagues at Cold Spring Harbor and Harvard (Efstratiadis Kafatos, Maxam and Maniatis, *Cell*, February, 1976) have chosen a somewhat simpler technique to make full-length double-strand cDNA once again using rabbit globin mRNA as the starting material. Single-strand cDNA is first prepared with reverse transcriptase, and this acts as a template for a second strand made with DNA polymerase, even in the absence of added primer. The double-strand cDNA was shown to be a single polynucleotide chain, looped round at the end, the primer being part of the original single-strand cDNA. The loop can be cut with single-strand specific S1-nuclease and each of the strands elongated with terminal transferase. Plasmid recombinants containing both α- and β-globin gene sequences were obtained. Full experimental details of the recombination and transformation are not given and will follow in a later paper, but it is likely that a poly(dA)–poly(dT) hybrid was used to link the cleaved plasmid and the globin gene fragment. Higuchi, Paddock and Salser, of the University of California, Los Angeles, have independently carried out similar experiments linking rabbit globin double-strand cDNA to plasmid pSC101 by poly(dA)–poly(dT) tails and have obtained at least 28 recombinants. They have been able to demonstrate in some cases that the inserted DNA truly codes for the globin sequence by the presence in this sequence of a specific R1 restriction endonuclease site shown to be present by this group's previous RNA sequence analysis.

Several British laboratories have also been interested in this technique, and the first person to report a positive result is Terry Rabbitts, of the MRC Molecular Biology Laboratory at Cambridge (this issue of *Nature*, page 221). He has used a different and elegant trick to integrate the gene. First, a related *E. coli* plasmid (mini-ColEI) was cut with restriction enzyme to give a linear molecule as before, and then oligo(dT) added directly to the cut ends. Rabbit globin mRNA binds to this oligo(dT) by its poly(A) tail, and the cDNA was synthesised with reverse transcriptase directly onto the plasmid. Poly(dT) tails were again added, this time to the globin cDNA. The mini-ColEIs containing globin cDNA were melted and reannealed with plasmids containing no globin cDNA but with a poly(dA) tail. Circles form between elongated cDNA-containing plasmids (containing poly(dT)) and strands from those with poly(dA) and when these infect *E. coli*, once again the bacterium repairs them, seals them up and the plasmids replicate normally.

Maniatis, Rabbitts and Salser all finish with a piece of rabbit globin gene bound covalently to plasmid by two oligo(dA)–oligo(dT) stretches, which cannot be cut by an available enzyme as neatly and easily as Rougeon's R1-sites. dA–dT hybrids are less stable than normal DNA double strands, and when the temperature goes up or the salt concentration goes down, they melt first, and form little single-strand bubbles while both inserted globin gene and original bacterial plasmid remain annealed. Therefore they can be broken by nucleases (such as S1) that attack single-stranded DNA preferentially. While not as elegant as Rougeon's technique for removing the inserted DNA, this should prove quite as effective.

One worry of bacterial geneticists has been stability of these recombinants —will they keep growing in culture, or will the inserted gene be eliminated? The two sequences (whether oligo(dA)–oligo(dT), or R1 sites) at either side of the insert could provide an active cross-over site for recombination with elimination of the mammalian DNA. Since the newly made recombinant grows more slowly than the original plasmid, any revertants would outgrow the carefully made hybrid plasmids. Fortunately, this does not seem to happen at a very fast rate, since the hybrids are stable for several months at least.

Ironically, only one of the four labs reporting so far is particularly interested in globin genes—several groups have already shown that α-globin or β-globin-specific cDNA can be prepared using other techniques, albeit rather tediously. Salser and his colleagues, however, should find the new technique very useful in their attempts to sequence globin mRNA. Both Rougeon and Rabbitts have been hoping to purify sequences complementary to mouse immunoglobulin mRNAs; Maniatis' colleagues hope to study the appearance of a complex series of mRNAs during insect development. The problem for all these groups in extending this technique is the purity of their mRNAs. In the case of immunoglobulin, it has proved very difficult to get mRNA greater than about 60% pure; the insect mRNAs are an even more complex mixture. Using genetic recombinants, scientists could now clone pure populations of double-strand cDNAs derived from each mRNA separately. Separating α- and β-globin genes represents a simple model system to this end. The fact that this has proved so simple will be very encouraging to people studying more complex systems.

The recent National Institutes of Health guidelines (outlined in part in *Nature*, **258**, 561; 1975) suggested that the containment facilities used should be mentioned by groups publishing in this field, to act as a guide to others. It is appropriate that one of these papers includes such a discussion—particularly as the guidelines are only just available. No doubt the full reports from the other groups, when published, will follow suit. The fact that the recommended safe procedures are followed for completely non-hazardous experiments should help to ensure that they are followed in the more delicate experiments which will no doubt be reported soon.

One last point—at the 'big' Asilomar meeting a year ago, several of us suggested that anyone obtaining a recombinant such as those discussed here should make it available to other laboratories as soon as the data on it have been published. This is because the major 'danger'—in this case virtually nil, but in future cases perhaps more substantial—arises during the first recombination and isolation of the plasmid containing the foreign genes, when the DNA integrated is not yet fully characterised. The power of these techniques is such that many others will be tempted to follow on immediately, perhaps with less experience and poorer facilities. I only hope that Rougeon, Rabbitts, Maniatis, Salser and their co-workers and followers will make their recombinants freely available. In this field, the way we start will determine whether others will act responsibly later. ☐

Great step forward for multiple sclerosis research

from Cedric Mims

RESEARCH into multiple sclerosis (MS) has been in a somewhat rejuvenated, exciting state ever since the discovery 10 years ago that two uncommon diseases of the central nervous system of man, subacute sclerosing panencephalitis and progressive multifocal leucoencephalopathy were both caused by viruses (measles and a new human papovavirus). At about the same time two other rare neurological diseases, Kuru and Creutzfeld–Jacob disease were found to be infectious in origin, and transmissible to subhuman primates. The causative agents in this case were unique virus-sized microorganisms, very similar to scrapie, and the incubation period was exceedingly long, occupying a large fraction of a life time. Earlier epidemiological studies had pointed to a possible infectious origin for MS, and these findings stimulated a serious hunt for a virus.

For some years a favourite candidate for the MS virus has been measles, but the evidence has been indirect, based on the high titres of antibody to measles found in the blood and cerebrospinal fluid of patients with MS. But not all patients have raised antibodies to measles, and antibodies to viruses such as mumps and rubella may also be elevated (Norrby *et al.*, *Infect. Immun*, **10**, 688; 1974). The association of MS with certain histocompatibility types (HLA 3 and 7) has prompted various virological suggestions. Perhaps the histocompatibility antigens on the cell surface act as virus receptors and thus promote susceptibility to MS, or perhaps certain immune response genes linked to these histocompatibility genes determine the immune response to a virus responsible for MS. A recent modest sized survey from Canada, however (Lamoureux *et al.*, *Brit. med. J.*, Jan 24, 183; 1976) indicates that patients with MS have unusual antibody responses to a variety of different antigens, antibody levels to certain viruses and to tetanus and diphtheria being lower than normal.

There have been a number of false alarms, virologically speaking, and when Dick Carp and his colleagues from the Institute for Research in Mental Retardation, Staten Island, New York, reported on a transmissible factor present in the brains and serum of MS patients (*J. exp. Med.*, **136**, 618; 1972), this was greeted with some scepticism, especially because the transmissible factor could only be demonstrated by its ability to decrease the number of circulating polymorphonuclear leukocytes in the blood of infected mice. The agent was titrated, and MS brains were found to have about 10^{12} infectious units per gram; it passed 50 but not 25 nm filters, and was serially transmissible in mice. Although it replicated after injection into mice there were no other signs of infection. But the test for a fall in circulating polymorphs was very difficult to standardise. These cells are notoriously variable in number, often difficult to identify in mouse blood films, and other laboratories were unable to confirm the findings. Carp then found that the same transmissible agent when added to a line of cultured mouse fibroblasts, caused a slowing of the rate of cell division, and this effect could be titrated out and the agent serially passaged through the cultures (*Infect. Immun.*, **9**, 1011; 1974). He later extended these findings, and described reduced cell yields of cultures after inoculation with various MS tissues, including brain, spleen, serum and cerebrospinal fluid (Carp *et al.*, *Neurology*, **25**, 492; 1975). As if to further strain our credulity his laboratory had reported that brains from mice infected with scrapie also caused a lowering of circulating polymorphs in mice (*Infect. Immun.*, **6**, 370; 1972). Titres were high and by diluting out his inocula, he was able to separate the polymorph lowering agent from scrapie itself (*J. infect. Dis.*, **128**, 256; 1973). The polymorph lowering effect was confirmed with scrapie from a variety of sources in a publication from Dickinson's laboratory in Edinburgh (*Nature*, **248**, 510; 1974). So far there have been no reports of reduced cell yields in tissue cultures inoculated with the scrapie-associated agent.

The status of all this work was raised dramatically by the recent publication of two papers from the Henles' laboratory in Philadelphia (Koldovsky *et al.*, *Infect. Immun.*, **12**, 1355; 1975; Henle *et al.*, *ibid*, **12**, 1367; 1975). They too found that the brains and serum from MS patients contain an agent that depresses the polymorph counts of mice. The effect was seen not only in all strains of mice tested, but also in rats, hamsters and guinea pigs. Titres were up to 10^9 infectious doses per gram in MS brain, and up to 10^3 in MS serum. The multiple sclerosis-associated agent (MSAA) was transmissible from animal to animal and passed through 50 nm but not 25 nm millipore filters. Perhaps the most important and convincing addition to Carp's work was the demonstration that nearly all MS sera and cerebrospinal fluids contained antibodies that neutralised the polymorph lowering effect. Many of the MS patients' relatives and nursing personnel also had serum antibodies, whereas only 1 of 59 sera from people without MS contacts had antibodies. Interestingly, 37 of 68 sera from East Africa were positive.

The evidence, therefore, is for a new microorganism infecting man, somewhere between poliomyelitis and polyoma virus in size, of unknown structure and nucleic acid, and strikingly associated with multiple sclerosis. Although the agent is present in large amounts in MS brains, and should be 25 to 50 nm in diameter, it has so far not been seen in the electron microscope. The long and mostly fruitless searches for virus-like bodies in MS brains make it seem unlikely that it will turn out to be a regularly sized or shaped object.

The stage is now set for intensive investigation of this new microorganism. It is especially to be hoped that there will be confirmation of Carp's report that the agent replicates and causes detectable changes in cultured cells. Strictly speaking the MSAA could turn out to be merely associated with some as yet undiscovered agent more directly responsible for MS, in much the same way that the other polymorph-lowering agent (possibly related to the MSAA) could be separated from scrapie. In recent years scrapie and MS have seemed to have been drifting away from each other, but there is much in these recent findings to suggest a closer association. Perhaps the MSAA is also membrane-associated as suggested by the susceptibility of both MSAA and scrapie to periodate. If both of these agents are mere membrane fragments or vesicles and of nondescript structure, then the failure so far to demonstrate either by electron microscopy is more understandable. One important difference, however, is that scrapie gives no sign of inducing the faintest flicker of an immune response in the infected host, whereas specific antibodies seem to be formed to the MSAA. If we accept the work on the scrapie-associated agent, scrapie becomes much more relevant for MS, reminding us that medical virologists neglect the field of veterinary virology at their peril.

Even if multiple sclerosis is an infectious disease, it is still vital to study the "soil" (host) as well as the "seed" (virus). Biochemical, cell culture, immunological and other investigations have been of great importance in MS research and they can now be oriented towards the associated microorganism. There is evidence for a strong immunological component in the disease process, and sophisticated immunological techniques can be brought to bear on this matter now that antigens of a specific microorganism seem to be available.

A hardened investigator might well at this stage still have some doubts and reservations about all this work, but it has given a fresh transfusion of life and hope to MS research. Evidently the new agent poses formidable technical problems, but it will nevertheless be further investigated and characterised, while serological studies should help to provide us with some understanding of its epidemiological behaviour. Finally if the agent proves to be causative rather than merely associated with MS, a vaccine could perhaps be produced to prevent infection and thus prevent the disease. □

Transferring nitrogen fixation genes

from J. E. Beringer

DURING the past few years considerable interest has centred on *in vitro* methods of joining together fragments of DNA from different sources, and the advantages and disadvantages of what is now called 'genetic engineering' have been the subject of much debate. This debate has tended to obscure the fact that some plasmids, such as the *Escherichia coli* sex factor F, have long been known to be able to pick up chromosomal DNA *in vivo* forming F′ factors. It is timely, therefore, that two papers in this issue of *Nature* (pages 268, 271) should remind us of the type of genetic manipulation that can be done *in vivo* and also show that such techniques are not only academically interesting, but have a real use—in this case to facilitate genetic studies of nitrogen-fixation genes. These papers by Dixon, Cannon and Kondorosi, and Cannon and Postgate describe first the transfer of *Klebsiella pneumoniae* nitrogen-fixation (Nif) genes from an F′, which has a very limited host range, to the plasmid RP4, which has a very wide host range, and then report on the transfer and functioning of these genes in such diverse bacteria as *K. pneumoniae*, *Rhizobium meliloti* and *Azotobacter vinelandii.*

Two particularly interesting points arise from these reports. The first concerns the transfer of this plasmid to *A. vinelandii.* Cannon and Postgate were able to show that the *Klebsiella* Nif genes it carries could overcome mutations in *A. vinelandii* that have resulted in the loss of either component I or component II activity of the enzyme nitrogenase. Whether functional hybrid nitrogenases were formed in *A. vinelandii* remains to be demonstrated. If this is indeed the case, and it also applies for other Gram-negative nitrogen fixers, then genetic studies of the *Klebsiella* Nif genes will not only provide a model for studies of nitrogen-fixation genes, but may also enable one to produce a range of plasmids carrying mutations in known Nif genes that can be used for complementation studies of these genes in other bacteria. Furthermore, studies of the transfer and expression of the *Klebsiella* Nif genes in a range of nitrogen-fixing and non-fixing bacteria should help to answer some of the simplest questions that can be asked about the feasibility of performing wide intergeneric transfers of nitrogen-fixation genes. In this respect the observation that transfer to *Pseudomonas aeruginosa* did not occur is slightly discouraging, since RP4 (the parent plasmid) was isolated in this organism and a derivative of it carrying the *E. coli* histidine operon produced by Olsen and Gonzalez (*Biochem. biophys. Res. Commun.*, **59**, 377; 1974) is both transmissible to, and stable in, *P. aeruginosa* The other point concerns the formation of the RP4-Nif plasmid. This was made in *E. coli* as the result of recombination between an F′ Nif previously produced by Cannon, Dixon and Postgate (*J. gen Microbiol.*, in the press) and the R factor RP4. In the present report Dixon, Cannon and Kondorosi suggest that this recombination occurred as a result of an interaction between identical translocating sequences carried on both plasmids These sequences, corresponding to the carbenicillin resistance determinant on RP4 (TnA) and the same resistance determinant on the F′, are capable of translocating on to a wide range of plasmids (Hedges and Jacob, *Molec. gen Genet*, **132**, 31; 1974). If the authors' suggestion that these translocating sequences can indeed facilitate recombination between plasmids that carry them, then they may well have demonstrated a general procedure for producing recombinant plasmids *in vivo*.

These studies would not have been possible without the availability of a group of R factors having the apparently unique property of being transferable to a very wide range of bacteria The R factors, which all belong to the incompatibility group P (Datta *et al.*, *J. Bact.*, **108**, 1244; 1971), first appeared in the late 1960s in isolates of *Pseudomonas aeruginosa* found to be highly resistant to carbenicillin, a particularly efficient semi-synthetic penicillin for use in pseudomonas infections. Studies of these pseudomonads soon showed that in many of the isolates resistance to carbenicillin was plasmid borne (that is, R-factor mediated) and linked to determinants for resistance to tetracycline and kanamycin. Although at the time a number of plasmids were known in *Pseudomonas*, R factor mediated drug resistance in these exceptionally antibiotic-resistant bacteria had not been reported. The most unusual property of these R factors, however, was their ability to transfer from *P. aeruginosa* to *E. coli* (Sykes and Richmond, *Nature*, **226**, 952; 1970). Although the publications that have followed this original observation suggest that all Gram-negative bacteria can act as recipients, as yet no report of transfer by conjugation or transformation to a

Membrane nearest neighbours

from Howard Goldfine and Alan F. Horwitz

ALTHOUGH the series of twelve papers describing the total synthesis of the structural gene for a tRNA precursor in the February 10th issue of the *Journal of Biological Chemistry* is undoubtedly the more impressive achievement, two other papers from the laboratory of H. Gobind Khorana may be of even greater interest to those concerned with membrane structure and function. These papers describe the synthesis of various photosensitive fatty acids and phospholipids and provide evidence that these compounds can be incorporated into natural biological membranes. These new analogues should prove useful as membrane probes to study previously inaccessible interactions between lipid and protein in the membrane.

Chakrabarti and Khorana (*Biochemistry*, **14**, 5021–5033; 1975) describe the relatively easy four-step synthesis of azidostearic acids from commercially available hydroxystearate isomers By standard synthetic methods they have put these and other photosensitive fatty acids on the middle carbon of the glycerophosphate backbones of phosphatidylcholine and phosphatidylethanolamine, both with palmitate esterified at the C-1 position. When the phospholipids were dispersed in an aqueous medium as sonicated vesicles and irradiated at 253 nm for 1 hour, extensive photolysis and cross linking occurred.

Greenberg, Chakrabarti and Khorana (*Proc natn. Acad Sci. U.S.A.*, **73**, 86–90; 1976) now report that an unsaturated fatty acid requiring mutant of *Escherichia coli* utilises the series of azidostearic acids with the azido group at various positions along the hydrocarbon chain. Most of these compounds were capable of supporting growth to the extent observed with the common unsaturated fatty acid, oleic acid. 12-Azido-oleic acid, a 16-azido *trans*-hexadecenoic acid, and two fatty acids with a bulkier 4-azido-2-nitrophenoxy group were also active, but the azidonitrophenoxy group on oleic acid did not permit growth at or above 25 °C. These studies show that the azido fatty acids are incorporated into cellular phospholipids to the extent usually observed with *cis*-monounsaturated fatty acids. Furthermore, they were incorporated largely at the middle carbon of the glycerophosphate backbone as is usually the case with unsaturated fatty acids. Since the photosensitive fatty acids behave like unsaturated fatty acids, the MIT group looks forward to being able to study the interactions of lipids with functional membrane proteins.

When irradiated the azido groups form highly reactive intermediates, which attach covalently to nearest neighbours. This cross linking should provide information on the amino acid residues in close apposition to phospholipids, and since the photosensitive group can be placed near either the carboxyl or methyl terminus of the fatty acid chains, it should be possible to determine the depth of penetration of proteins into the bilayer and to map the exposed amino acid residues as well.

Reconstitution of membrane proteins with specific photosensitive phospholipids provides an alternative means of determining such spatial relationships. The polar head group of the lipids is another potential site for lipid–protein interaction, and two affinity labels have already been synthesised by Chakrabarti and Khorana, in which a polar photoactivable group replaces ethanolamine.

Extension of their work with an *E. coli* mutant to a similar mutant of a *Pseudomonas* species is already in progress, and they visualise future work with *Neurospora* and yeast mutants and even with mammalian cells grown in fatty acid deficient media. Although not specifically mentioned, questions of lipid–lipid "nearest neighbours" and membrane lipid asymmetry can also be approached with these compounds in either natural membranes or artificial systems. One caution, however, is noted. The azido group absorbs weakly in the ultraviolet and there is obvious overlap with nucleic acid and protein absorption The use of radioactively tagged azido fatty acids may permit shorter irradiation times but other more photoreactive groups are being sought.

Gram-positive bacterium has appeared. Whether this represents a true difference in the ability of either group of bacteria to allow the replication and maintenance of plasmids from the other group, or simply that conjugation cannot occur and transformation has just not been reported, remains to be elucidated.

Future research using P-group plasmids carrying Nif or other genes looks promising, both for studies of gene function in different environments and for the genetic analysis of Gram-negative bacteria. However, problems of instability and the reduction in the host range of such plasmids, as reported by Dixon, Cannon and Kondorosi, will need to be overcome. Also it has yet to be demonstrated that the type of recombination event involved in the formation of the RP4-Nif plasmid will provide a general method for making R′ factors in the future. ☐

PETRA : come and help

from David J. Miller

PLANNING the future of European high-energy physics is not a very cheerful exercise just now, but there is one major ground for optimism, despite the budget squeezes which seem to be happening all round. The Germans have announced that they will go ahead rapidly with the construction of their electron–positron colliding-beam accelerator—PETRA. This machine, which should begin operations before its American equivalent, called PEP, is very similar to the EPIC project which Britain recently decided to abandon. It gives European physicists a chance to play a leading part in understanding the "new physics" which has emerged with the discovery of weak neutral currents and of the J/psi family of particles.

In deciding to build PETRA, Germany has shouldered a very heavy financial burden and it has been made clear that she cannot, by herself, afford to provide the large detection systems which such a machine will need. International collaborations are therefore being encouraged to bring equipment, expertise and manpower to join in the experimental programme. As a first houseparty to introduce possible collaborators to one another, the European Committee on Future Accelerators (ECFA) sponsored a meeting during the first week of March at the Frascati laboratory near Rome. It was attended by physicists from universities and laboratories throughout Europe, the United States and Japan. Theoretical predictions were reviewed, confirming the enormous range of worthwhile physics available to the machine; experimental techniques were considered, and the basic parameters of PETRA were presented. The meeting became more political during the later part of the week when people put forward specific ideas for experimental equipment, and possible collaborators began discreetly to have lunch together. Professor Schopper, head of the laboratory where PETRA will be built, warned of the three essential steps in setting up a "marriage" between collaborating groups. First it is necessary to recognise the beauty of the prospective partner—how good are their ideas? This is usually clear on brief aquaintance, but the second question is more delicate—do they have money, or rather, do their parents (their governments) have money? Finally, one has to judge whether the collaborating groups are likely to be compatible throughout three or four years of hectic collaboration, with many crises to be survived.

A large proportion of the first sketches of apparatus for PETRA looked almost identical. The classic general purpose detector has a superconducting solenoid magnet with diameter and length between $1\frac{1}{2}$ and 6 metres. It is filled inside with drift chambers to reconstruct charged-particle tracks coming from $e^+ e^-$ collisions, and it is surrounded by electron, photon and muon detectors. Other schemes were also suggested which laid greater stress on detecting electrons and photons in particular, or on muons, or on recognising the exact identity of strongly interacting particles. The British contingent, representing half-a-dozen universities and the Science Research Council laboratories, presented a general purpose solenoid detector which had been costed more carefully than many of the other systems discussed. The estimated price is about £2 million, and it is unlikely that all of this could be found from Britain alone since it would consume too large a fraction of the total national funds available for experiments at CERN and during the last years of NIMROD. In fact, none of the nations represented at the meeting seemed keen to pay for the whole of such a device by itself.

Courtships begun at Frascati must progress quickly. In order to have equipment ready for the first 15 GeV PETRA experiments in 1979 it will be necessary to start building the apparatus in the autumn of this year at the latest. The machine will eventually be able to accommodate six separate detectors, each enclosing a collision point of the two beams, but only two collision points are to be available at first so there will be strenuous competition to win one of these places. The British group, backed by the expertise of the Rutherford Laboratory in superconducting magnets, seemed to be among the more popular debutantes at Frascati. If the SRC is able to provide a respectable dowry, commensurate with expenditure on individual CERN experiments, then there is a good chance that a match can be made with one or more of the strong European groups, and that Britain will take part in $e^+ e^-$ physics in 1979 despite the loss of EPIC.

The strong, weak and electromagnetic interactions are so fundamental to all modern science and technology that we often behave as if we understood them, merely because we can use them. But major changes in our understanding are now taking place, and no-one doubts that results from both CERN and PETRA will be important in consolidating these ideas. European collaborations have played a major part in the first wave of the "new physics". Even in a time of economic troubles it would seem foolish for any government to allow its physicists to lose touch with this frontier of knowledge. ☐

New view of the cell cycle

from Robert Shields

THE period between successive cell mitoses is conventionally divided into four phases—G_1, the period between mitosis and the onset of DNA synthesis, S the period of DNA synthesis, G_2 the time between completion of DNA synthesis and the beginning of mitosis itself. Measurements of these phases show that whereas G_2, M and S are of relatively constant duration G_1 can attain widely different values depending on the conditions. Even within cell populations G_1 is usually highly variable in duration and the distribution of intermitotic times (times between successive mitoses) is invariably skewed towards longer time intervals. The conventional explanation for this skewness has been that it is some kind of reflection of heterogeneity in the cell population, but the reason why G_1 is much more variable than the rest of the cycle has never been adequately explained.

A new concept of the cell cycle has recently been proposed which not only predicts that cell cycle times must be heterogeneous but accounts quantitatively for their observed frequency distributions (Smith and Martin, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1263; 1973). The model divides the cell cycle into two parts. One termed the B phase is regarded as a deterministic and highly coordinated process; it corresponds to the conventional S, G_2, M and part of G_1 phase. The other part (contained within G_1) is called the A state and is a sort of limbo in which the cell is not progressing towards mitosis; the cell may remain in this state for any length of time subject to the condition that under steady state conditions the probability per unit time (termed transition probability) of the cells leaving the A state and entering the B phase is a constant. In other words, cells leave the A state exponentially with respect to the time they have spent in that state in much the same way as a radioactive material will decay at a rate determined by its half life. In a population of cells the shortest intermitotic time is then given by the cell that leaves the A state immediately it enters, intermitotic times greater than this minimum should be exponentially distributed. The theory can account satisfactorily for the distribution of intermitotic times in a wide variety of cell types from bacteria to animal cells. What is more remarkable is that the theory predicts that the difference in intermitotic times of sibling cells should also be exponentially distributed (Minor and Smith. *Nature*, **248**, 241; 1974). This is observed in practice.

In principle the transition probability can take any value, high values would mean that most of the cells spend very short times in the A state, have fairly short homogeneous intermitotic times and so grow relatively rapidly. Low values would mean that the G_1 period would in the main be long or equivalent to so-called G_0 periods postulated for slowly or non-proliferating cells. Viewed in this way the problem of changing growth rate reduces to one of changing transition probability.

The paper by Brooks in this issue of *Nature* (page 248) examines the relevance of transition probability to mitogenic stimulation by serum of so-called G_0 cells. Since the characteristics of this type of stimulation are shared by systems as diverse as lectin-stimulated lymphocytes and liver regeneration after partial hepatectomy, the experiments may be relevant to other systems as well. When serum is re-added to serum-starved fibroblasts there is a characteristic lag (which is independent of serum concentration) before any increase in DNA synthesis is seen. After this lag cells begin making DNA asynchronously. In a previous publication (*J. cell. Physiol.*, **86**, 369; 1975) Brooks showed that the apparent asynchrony in DNA synthesis was a result of the cells entering S phase with first order kinetics (that is exponentially), the actual rate constant depending on the concentration of serum used. Initiation of DNA synthesis can then be regarded as a random event occurring with a transition probability determined by the serum concentration. What serum does is to change abruptly the transition probability from the low value seen in quiescent fibroblasts to a higher value and so cells will leave the A state and enter the B phase (and hence DNA synthesis) at a faster rate.

The important aspect of these results is the observation that suboptimal doses of mitogen do not commit a subfraction of the population to enter DNA synthesis at a fixed time but cause the entire cell population to enter S phase with slower first order kinetics. The concept that a fraction of cells is committed by suboptimal doses of mitogen can now be seen as erroneous because it fails to take into account the kinetics of entry into S phase. Furthermore, the new value of transition probability produced by the serum is constant. This shows that mitogens such as serum do not trigger a sequence of events leading to DNA synthesis, for if they did, with increasing time after stimulation it would be more and more likely that the putative sequence was completed and hence the probability of entering DNA synthesis would increase with time. This is not the case.

In the present publication Brooks approaches the problem of the lag before any marked increase in DNA synthesis is seen. By following the kinetics of the entry into DNA synthesis after switching the serum concentrations from low to high values and *vice versa*, he comes to the conclusion that the lag after mitogenic stimulation represents the time required to effect any upward change in the transition probability regardless of its initial value and not the time taken to irreversibly commit all the cells to DNA synthesis.

It seems that the concept of there being a probabilistic transition within the cell cycle not only accounts for the heterogeneity of cell cycle times in a cell population, but positively predicts that such heterogeneity exists. Furthermore, the theory can account satisfactorily for the kinetics of entry of cells into DNA synthesis after mitogenic stimulation. The biochemical basis for the probabilistic transition remains obscure, however, but it is only through an appreciation of the dynamics of the cell cycle that its nature will be elucidated. ☐

Pop goes the weasel

from a Correspondent

MOST mammalian carnivores tend to be fairly choosy about what they eat when food is plentiful but rather less so when it is not. An ability to cope with a wide range of food species has a high survival value for weasels (*Mustela nivalis*) which depend on characteristically fluctuating rodent populations. Brown and Lasiewski (*Ecology*, **53**, 939; 1972) have drawn attention to the difference in size between males and females and to the energetic problems their shape causes. The long, thin body is costly to keep warm and to compensate, weasels have a metabolic rate between two and three times higher than that of other mustelids (Iverson, *J. cell. comp. Physiol.*, **81**, 341; 1972). Not only are they territorial, thus reducing intraspecific competition for food, but they quickly reduce their reproductive rate should food become scarce (Erlinge, *Oikos*, **25**, 308; 1974) and, additionally, demonstrate a special kind of prey switching behaviour (Erlinge, *Oikos*, **26**, 378; 1975).

In spruce woodland in southern Sweden, weasels feed on whatever prey is available. A slight seasonal change in diet is observed with fewer young rabbits taken during the winter when they are less abundant. Other changes broadly reflect fluctuations in the rodent populations. Erlinge's data indicate that during spring and summer, when rabbits are relatively abundant and rodents relatively scarce, male weasels, which weigh almost twice as much as females, switch their attention to rabbits, leaving more of the rodents available to the smaller females. Rabbits constitute 40% of the males' diet in spring, with *Microtus agrestis* accounting for 35%; at the same time of year the latter species constitutes 86% of the diet of female weasels and rabbits just 6%. Similarly, males prey quite heavily on the large vole *Arvicola terrestris* and leave the bank vole *Clethrionomys glareolus* for the females. During autumn when rodents are abundant such segregation does not occur. Although Erlinge has shown that weasel populations are sometimes limited by food shortage, prey switching presumably allows the persistence of higher overall numbers.

British weasels are larger than their Swedish cousins but still show a marked difference between the sexes. Their catholic approach towards food has been demonstrated most recently by Moors who observed their feeding habits on two farmland study areas in north east Scotland (*J. Zool., Lond.*, **177,** 455; 1975), and found a similar situation to that in Sweden. *Microtus agrestis* and rabbits were particularly favoured items. But there was one marked difference between the studies which is as interesting as it is difficult to interpret. *Clethrionomys glareolus*, highly favoured by the Swedish female weasels was almost totally shunned by Scottish weasels of both sexes, yet on one of the two study areas bank voles were actually more abundant than *Microtus agrestis*. The little work done on the diet of British weasels does suggest that nowhere is *Clethrionomys* a food species of major importance, although it is very abundant (Day, *J. Zool.*, **155,** 485; 1968, Walker, *J. Zool.*, **166,** 474; 1972). Are British habitats in general and Scottish farmland in particular that much more favourable to weasels than southern Swedish spruce forests in producing sufficient numbers of the favoured and, perhaps, the less abundant rodent species? If they do, the size differential which has been shown to facilitate prey switching behaviour in Sweden is of lesser survival value to British populations. It may, however, enhance survival in years when all species of rodent are in decline. Further studies on weasel feeding ecology will be of the greatest interest to population ecologists and evolutionary strategists alike. □

A hundred years ago

THE general staff of the German empire has published a report of experiments made in Germany on ballooning at the expense of the Imperial Government. The conclusions throw no new light on the subject, but the German officers believe that the mechanical direction of balloons is by no means an impossibility. They even suppose that the problem of ascending or descending without using ballast or the valve, is very likely to receive a speedy solution. They propose to the Government to determine by means of experiments what is the best diameter for the helix when it is applied to a balloon of a certain capacity. They propose also to try the efficacy of wings for propelling balloons. They are not of the common opinion that the diameter of balloons can be indefinitely enlarged.

from *Nature*, **13,** March 16, 397; 1876

Galactic gas dynamics

A meeting on Galactic Gas Dynamics was held by the Royal Astronomical Society in London on February 13, 1976.

from a Correspondent

THE principles of fluid dynamics can be applied with quite remarkable generality to problems in astrophysics. At the meeting astronomers learnt of the great versatility of gas dynamics in coping with the varied conditions in astrophysical plasmas. Within the basic theme of Galactic Gas Dynamics, we saw how ideas of fluid flow have been applied to plasmas with vastly different densities and scale sizes, from hot gas clouds occupying clusters of galaxies, to the problems of star formation.

Astrophysicists have long been intrigued by how such well-defined and apparently long lived spiral structures such as our own galaxy can exist without winding up in a disk of gas and stars that is rotating faster in its central regions than at the edge. The modern view is that these spiral patterns, or density waves, may persist as stars and gas move through them, and that a gravitational torque is developed, continually driving angular momentum outwards from the centre of the galaxy. According to V. Icke (Institute of Astronomy, Cambridge), gas has a particularly important role in the transport of this angular momentum. The more gas in a galaxy, the easier this transport becomes and a relatively open spiral results. He believes that this is the probable cause of the observed correlation between the proportion of gas in a galaxy and its classification in the famous Hubble Sequence of spiral galaxies.

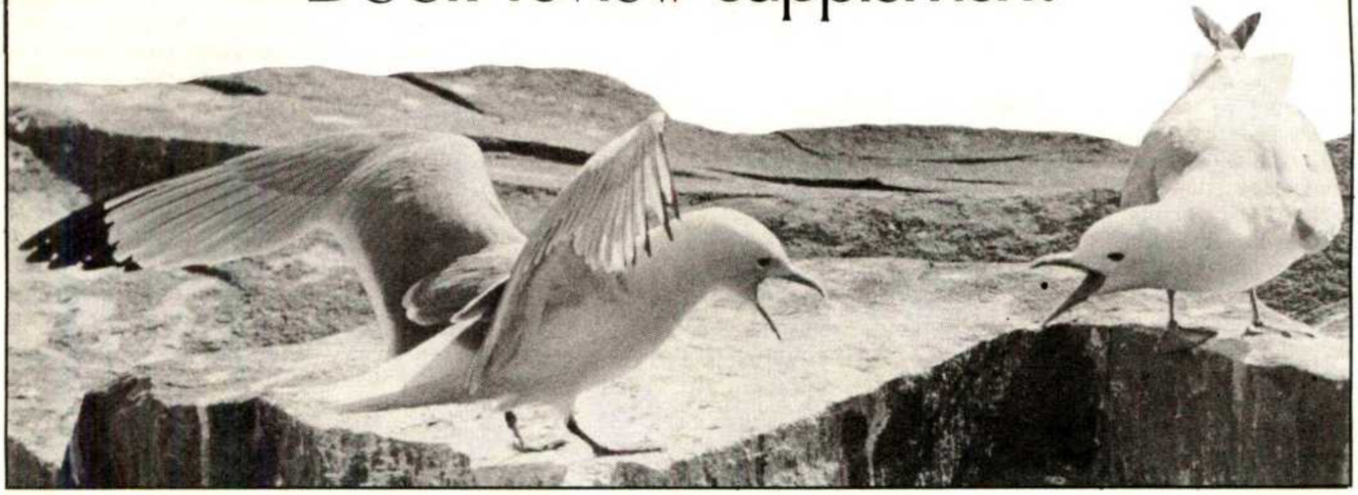
T. Matsuda (University College, Cardiff) then spoke on the case of the peculiar 'barred' spirals, in which a rod-like structure or 'bar' is seen in the central regions. He considers that the complications introduced by this asymmetrical potential make the problem into a case for numerical techniques. The resulting computed flow patterns show that a suitably shaped bar can indeed set up a two-armed spiral flow, with shock fronts that agree well in position with regions of star formation seen in actual galaxies. What did not emerge from the calculations, however, was why such a bar should have formed in the first place.

The afternoon was devoted to smaller scale galactic phenomena, the formation of stars and HII regions and the interaction of supernovae with the interstellar medium. With the recent widespread use of numerical techniques, F. Goldsworthy (University of Leeds) sounded a cautionary note with regard to the treatment of ionisation fronts, the sharp boundaries between neutral and ionised hydrogen surrounding young, hot stars. Unlike the relatively simple shock fronts that occur in ordinary gas dynamics, an ionisation front can be of two species, each with two strengths, and one of these, the strong 'deflagration' front, is characterised by an inherent indeterminacy difficult to overcome in computer solutions. Goldsworthy reported on a noble attempt to exorcise this particular devil by means of a scheme that at least manages to avoid its invocation.

S. Falle (University of Manchester) made a similar point about the usual numerical method of treating shocks by including an artificial viscosity term. He showed that this method can be greatly in error if used to estimate the luminosity of the radiating shocks that occur in the late stages of supernovae. By using more accurate shock-fitting techniques, Falle has so far managed to distinguish five separate types of old supernova remnant. On the other hand, F. Kahn (University of Manchester) was prepared to use an analytical approximation in his treatment of this subject, and reported that very substantial amounts of hot gas must exist in a supernova remnant, even a million years after the supernova explosion. At this late stage remnants are likely to bump into, and coalesce with, their nearest neighbours in the Galactic plane. He calculates that about 30% of the Galactic gas is kept hot in this way.

As the meeting progressed it became clear that an impressive array of numerical techniques is now available to tackle these gas dynamical problems. During the day the results were shown from two massive computer codes using two spatial dimensions and no less than four one-dimensional programs appeared. But the talk by A. Nelson (University College, Cardiff), who was concerned with the small scale warping or corrugation of gas in the Galactic plane, served as an illustration of one of the cardinal rules of gas dynamics. Fluid flows, no matter how symmetrical they might appear, are inevitably three-dimensional, and it will still be some time before numerical methods can catch up in this tricky subject. □

Book review supplement



Male Kittiwakes contesting a cliff nest site Photo: Niko Tinbergen

Analysis of function

John R. Krebs

Function and Evolution in Behaviour. (Essays in Honour of Professor Niko Tinbergen, FRS.) Edited by Gerard Baerends, Colin Beer and Aubrey Manning. Pp. xxxi+393. (Clarendon: Oxford, Oxford University: London, February 1976.) £16.50 net.

IN his introduction to a volume of Tinbergen's collected works published a few years ago, Sir Peter Medawar recounted the story of an eminent neurophysiologist who said of ethology "Why, that's just bird-watching, isn't it?". The point of the story is, of course, that Niko Tinbergen has done more than any other single person to transform the biological study of behaviour from descriptive natural history into a hard experimental science. After emphasising description as a starting point, Tinbergen and his associates have succeeded in carrying out simple, but rigorous and incisive, experiments in the laboratory and field to elucidate problems ranging from why seagulls are white, to how digger wasps find their nests.

Five years ago, I think that most ethologists would have agreed that the most exciting developments in what might broadly count as ethology involved the meeting point of ethological and neurophysiological research—for example, in the mapping of neural pathways underlying simple behaviour patterns in molluscs. In the last year or two, however, things have changed, and there has been a dramatic upsurge of interest in the area in which Tinbergen made some of his most important contributions. We used to call it behavioural ecology, but now it is known as sociobiology. Sociobiology (as defined by the contents of E. O. Wilson's book) involves much more than the work of Tinbergen and his students, but the studies carried out by his group are without doubt among the best in elucidating how natural selection acts to mould the social organisation of a species. Work on the adaptive significance of colonial nesting in gulls, and on the social organisation of carnivores are examples which spring to mind as typifying the blend of ecology, behaviour and evolution which characterises much of the work of the Tinbergen group.

In the past, ethologists as a whole can be faulted for having been somewhat careless in thinking about how natural selection influences behaviour, and it is a pity to see the confusion continue in this volume dedicated to one who thinks so clearly about the problem. There seem to have been three types of difficulty in the literature dealing with the function or survival value of behaviour, all of which occur in this volume of essays. The first, and most widely appreciated, is the old Panglossian fallacy that natural selection favours adaptations that are good for the species as a whole, rather than acting at the level of the individual. This type of argument crops up in the article by Immelmann on early learning—he suggests that imprinting is advantageous because it enables a species to evolve rapidly—as well as in the article by Baerends in which he makes a similar case for the advantage of flexible motivational control displays. The second 'function' fallacy is discussed at length in articles by Hinde and Beer. It is partly a semantic issue arising from the oft-posed question "What is the function of such-and-such behaviour?", which is of course shorthand for asking why natural selection, the differential survival of genotypes, favours that particular behaviour. The problem with this question is, as Beer and Hinde point out, that most behaviour patterns (or structures) are likely to have several functions. Territorial behaviour may increase fitness because it preserves a food supply near the nest, it reduces the chance of predation and so on. The confusion arises when one tries to ask which function came first, an historical question which cannot be answered by 'survival value' experiments. Sometimes it is possible to deduce which function came first on logical grounds or on features of design. Thus one can conclude that any population-limiting effect of territorial behaviour is likely to be secondary; and the design of a reddish egret's (*Dichromanassa rufescens*) wing suggests it is primarily a locomotor organ, although it may be held outstretched in feeding, to cut down reflection from the water surface. The use of its wing in feeding, however, increases a reddish egret's chance of survival, so this has to be counted as one of the functions of wings. It is difficult to see how one can distinguish, as Hinde claims in his essay, between 'functions' and 'biologically advantageous consequences which change reproductive success'. It is clear that function is, to quote G. C. Williams, an "onerous concept", so perhaps the best solution would be to rephrase the question mentioned above as "How does such-and-such behaviour influence fitness?".

The third difficulty in the analysis of function is that ethologists often make the implicit assumption that behaviour is more responsive than are other features of an animal to the action of natural selection. To give an example, it is a widely accepted ethological fact that many courtship and threat displays in which the participants suddenly face away from each other, serve the function of cutting off stimulating visual input and so allow the animals to control their motivational state. Another familiar example is the 'Fraser–Darling Effect': the idea that colonial nesting and communal displays function to enhance gonad growth through mutual stimulation. Both of these arguments imply that natural selection is not very good at designing animals with efficient physiological systems, so that it has to call on behavioural adaptations to sort things out. This is not to say that the cut-off hypothesis is wrong, but without careful thought it is all too easy for the ethologist to dream up glib functional explanations for behaviour by assuming that other features of the animal are not subject to natural selection as well.

In addition to the two general discussions of function, to which I have already referred, the book contains several more specific studies of the effect of natural selection on behaviour. Of special note are the papers by Liley and Seghers who show how predation pressure influences morphology and behaviour in guppies, Kruuk on the relationship between social organisation and feeding habits in carnivores, and Patterson's attempt to disentangle the factors influencing fighting in rook flocks. Baerends reviews the literature on the motivational and evolutionary origin of displays, and the overall impression is that little progress has been made since Tinbergen wrote his major paper on this subject in 1959. Although it does not seem to have filtered through to the ethological community, I think it likely that the recent game theory analysis of ritualised conflicts, by Maynard Smith and Parker, will provide a starting point for totally new insights into the nature of courtship and threat displays.

Without listing the contents in detail, I think it is fair to say that, although there are good discussions of various areas of important progress in ethology as a whole, the book does not reflect the exciting new developments—for example, in the quantitative analysis of function—which have arisen specifically out of the lines of research initiated by Tinbergen himself. Perhaps by including contributions from some of Tinbergen's recent students and collaborators, the volume could have given a better impression how his particular inspiration is as much as ever at the very forefront of ethological research.

Description before analysis

Roger French

The Great Instauration: Science, Medicine and Reform, 1626–1660. By Charles Webster. Pp. xvi+630. (Duckworth: London, November 1975.) £13.50.

"THE history of science has predominantly assumed the character of an index of progress towards our present intellectual position". With these opening words, the author proceeds to justify an examination of science in seventeenth century England, and to criticise the growth of a rather whiggish internalism in historical accounts of the sciences. Now, one of the reasons for regarding history of science, and in particular, history of medicine, as an independent discipline, is because the evidence to which the historian first turns his attention is of the same nature as that presented to the figures he is studying—that is, textual evidence—whereas the historian-at-large is faced rather more often with written evidence of something that is not documentary, like a political action. The directness, continuity and the subject matter itself of the former lead naturally to a history-of-ideas approach, whereas the inferential nature of the latter directs the attention to the historical circumstances of the object of study. Those with an interest in the history of science know that the historian of ideas can sometimes neglect every other historical factor, and that on the other hand a circumstantial history can degenerate into the merest sociology. By and large, history of medicine at least is underworked. We should resist the temptation to frame hypotheses and find causes in history before we are done with the texts that furnish us with the historical evidence. Description should precede analysis.

Robert Boyle (1627–91), a seventeenth century English natural philosopher

Ronan Picture Library

The study of seventeenth century English science has many pitfalls of this kind. Although his book is 'history-at-large', Webster falls into none of them. He makes us aware that historical circumstances in the sense used above can be intellectual, and the plan of his book is to reveal the progress of the *idea* that man can restore himself to the state of knowledge of, and command over, nature that he possessed before the Fall. In working out this idea, attention was given to education, to agriculture, to public health, and above all to Reform. Webster examines in considerable detail the social, political and religious groupings of the exponents of these ideas in a way that adds another dimension to the more usual and more technical accounts of the Baconians and Harveians of the English seventeenth century. So consistently is this approach followed that it disarms the critic who might expect a technical exposition of Harvey's work in a book with this title. Webster also reminds us that description should precede analysis in not attempting too great a correlation between the results of the internal and external approaches. It would be nice to know why Culpeper felt that his reforming zeal could best be served by translating the traditional, rather scholastic and largely discredited *Ars Medica* of Galen. Indeed, Culpeper's whole notion of medicine was Galenic, as was that of the College of Physicians whose monopoly he attacked. It would also be nice to know more precisely the relationships between the professional institutions of the physicians on one hand and on the other the internal changes in Galenism that led to its retention as a doctrine, or to its collapse. Webster's exploration of the intellectual and social context of the 'most intrinsically interesting' scientific developments, the examination of 'the worldview of a particular society' is pursued without the distortion caused by the spectacles of modern science, but with a wealth of detail that will make the book invaluable to historians of all shades and the natural authority for those working in the period. • ☐

Riding roughshod over cancer research

John Cairns

The Biology of Cancer: A New Approach. By P. R. J. Burch. Pp. 452. (MTP Lancaster, January 1976.) £11.50, $23.23.

PROFESSOR BURCH has recently acquired a certain fame thanks to his much publicised assertion that cigarette smoking is not a cause of lung cancer. This is just one consequence of his theory that most diseases are largely determined by random events within us that owe nothing to external agents in our environment. He has now written a detailed account (one might call it a polemical tract) describing how he came to his present, unorthodox position.

The argument runs as follows: Over most of our lifespan the incidence of the common cancers rises roughly as the fifth power of age. This is usually explained by saying that cancer is the end result of several independent steps (mutations) whose frequency among the cells at risk accumulates linearly with time. On further scrutiny it turns out, however, that many other diseases—ranging from arteriosclerosis and osteoarthritis to certain forms of psychosis—show a similar relationship to age. As these diseases are clearly multifocal in origin, they can hardly be ascribed to a particular set of rare mutations having occurred in one of the cells at risk in artery, joint, brain, and so on. If we want to persist with multistep models for disease, we should therefore try to come up with some formulation that allows a set of mutations in one cell to be the cause of unifocal or multifocal changes among other cells. So let us postulate that the main tissues of the body are divided up into a large number of subsets containing a few hundred cells whose growth is controlled by a central system of cells divided into a similar number of subsets, and let us postulate that most diseases are due to defects in the central controlling cells; unifocal diseases like cancer are due to mutations that fall within a single subset of controlling cells; multifocal diseases like arteriosclerosis are due to mutations in the primordial stem cells which are the parents for many subsets of controlling cells. Burch goes on to suggest that the central controlling cells are lymphocytes, that the dialogue between central and peripheral systems is conducted by means of pairs of specifically interacting molecules, and that these molecules are coded for by genes which contain palindromic base sequences and therefore are apt to be read backwards as well as forwards.

Professor Burch. Photo: Leeds University

The theory owes an acknowledged debt to such notions as the control of growth by 'chalones', the recognition of self and not-self, the creation of 'forbidden clones' and the control of cancer by immune surveillance—ideas championed by many people, Burnet in particular, at one time or another. As formulated by Burch the theory provides a plethora of independent variables—the mutation rates for the particular genes involved, the number of genes that must be altered to inactivate an appropriate central controlling cell, the number of such cells that have to be affected, the proportion of people that are genetically susceptible, and so on. Because some of the functions that can be generated with the model do not increase steadily with time but go through a maximum, the theory can be made to take in even such things as the age distribution of a childhood disease like measles (for me, the clinching *reductio ad absurdum*). Further, as Burch modestly points out, "so far as I am aware, our theory is the only one that offers a general solution to the problem of the anatomical specificity of the lesions of natural disease".

Now, all this curve fitting would be just innocent fun, albeit of a rather old-fashioned kind, were it not that somehow the idea of chance events scattered randomly among central controlling elements has been taken to mean that the events cannot be due to external agents. I must say I do not understand the logic here; after all, one of the first collections of biological data shown to follow the Poisson distribution was the number of men killed by horses each year in the Prussian army, and there the external agent was plain for all to see, on the hoof as it were. Anyway, that is what Burch believes and so he sets out to show that the incidence of the common cancers is not perceptibly influenced by environment and therefore that nothing can be done to prevent cancer, or most other diseases if it comes to that. (I might sympathise with this attitude if he were an old scientist subconsciously wishing to make the virtues and rewards of science decline to zero coincidentally with his retirement, but Burch is hale and hearty as far as I know and has no reason to go round preaching a kind of molecular nihilism).

Obviously the theory rides roughshod over most forms of cancer research. For example, the connection between external agent and susceptible cell is too direct to be denied for the various experimental cancers in animals and for most industrial cancers, and so Burch is forced to say that these are exceptional and have no bearing on the common 'natural' cancers. As for the copious epidemiological evidence that the incidence of the common cancers changes with alterations in habits and environment, he argues for a combination of illusion and conspiracy—for example, Japanese migrants to the US show altered cancer rates because they have a genetic constitution that drives them to migrate to a country where coincidentally the population suffers the same spectrum of cancers as they themselves; similarly, the apparent steady decline in stomach cancer and rise in lung cancer over the past 50 years are artefacts due to changes in diagnostic fashions.

Now, I believe that all this is what might euphemistically be called wrong. Indeed, I think that even Burch will come round to that opinion. Were the biology of cancer a normal branch of science, his unorthodox beliefs might be stimulating and would add a certain piquancy to the lives of cancer research workers, like the pleasure one gets on meeting someone who really believes the earth is flat. Unfortunately the biology of cancer is not that kind of subject. This book will undoubtedly persuade many people to continue smoking who might otherwise have stopped, and may confuse those who legislate on the search for and control of carcinogens in our environment. Some of these people will, I am sure, have been persuaded not by actually reading the book and being swayed by the inexorable logic of his argument, but simply by the thought that when a professor writes a fat, obscurely mathematical tome on any subject whatsoever, there must be something in what he is saying. And that is why I wish this book had not been written. □

Genial corrective

A. R. Ubbelohde

The Royal Society and Its Dining Clubs. By T. E. Allibone. Pp. xv+457. (Pergamon: Oxford and New York, 1976.) £10.

EFFECTIVE pursuit of natural science demands much concentration. Dr Johnson once compared a course of chemistry in this respect with a course in walking the tight-rope, as a cure for Boswell's melancholy. Clearly, a Dining Club provides genial correctives for any excessive abstraction scientists may have to practice. This adds a distinctive relish to the long history of 'clubbable' Fellows of the Royal Society dining together. Dr Allibone's narrative is the present successor to earlier chronicles of the Royal Society's Dining Clubs. Two of these histories, by Sir Archibald Geikie and Dr Bonney only stretch down to 1901, so that Dr Allibone has had to grapple with some most memorable events in science as well as in world affairs. From his 'history' it is entertaining and fascinating to mark the uneven penetration of such events into the after-dinner discussions of the Club.

At the present time, the club numbers rather less than 100 members. Only a fraction come to dine on any one evening. It is also the custom for individual members of the Club to bring distinguished visitors, often from overseas, to a dinner. Quite often the President of the Royal Society is in the chair. Under the Chairman's guidance the talk ranges informally, over narrow specialist interests as well as widely in relation to the world at large. Week by week and year by year the Senior Treasurer for that year keeps records of those present, their dinner menu, and the heads of discussion, for storage in the club archives, which are kept private until the next history is due to be written.

Much praise is due to Dr Allibone for the way in which he has tackled this tremendous task. He makes it clear how formal continuity of the present Club can be traced back to 1743; but the Club can claim plausible links with earlier diners up to 1645. Samuel Pepys records his evident satisfaction with the fare as well as the discourse in the seventeenth century. Now, the club fare has become decidedly 'plainer' even if our thinking is 'higher'. It may be some mitigation too of any euphoria at present meetings of the Royal Society that dinners of 'The Club' now follow these, instead of preceding them as in Pepys' time.

With over 200 years of continuous archives to deal with, the present history has perforce concentrated much information in the form of nourishing pemmican, or sometimes by presenting well articulated skeletons from amongst the scientific worthies of the past. The long list of famous names cited makes the book valuable as well as fascinating. Different readers will probably let their imagination seize on different personalities to clothe, and Dr Allibone is to be congratulated that the choice provided is large. Thus it is striking to find that Henry Cavendish, who is often pictured as a millionaire scientific misanthrope, was one of the most assiduous diners. Clearly, a 'clubbable' man of his day. The colourful personality of Sir Joseph Banks receives rather lurid light from the record of his having purchased a silver bowl holding a gallon of punch and also of his having led a breakaway dining club into schism. When later he was elected President of the Royal Society, however, the schism was gradually healed. Then, too, there is the rather sad little note that Lavoisier in 1786 established a monthly dining club of scientists, the 'Philomatic Society', almost under the shadow of the guillotine.

This does raise the question (with one eye on Dr Johnson and perhaps one on Mr Pickwick) whether elite dining clubs (however chosen) are truly congenial only to the British temperament. One may hope that modern conditions of living will never extirpate such an amiable weakness or custom. So far as the Royal Society dining club is concerned, Dr Allibone's history will certainly help to perpetuate its remarkable longevity. □

Authentication in art

Harold Barker

Authenticity in Art: The Scientific Detection of Forgery. By Stuart J. Fleming. Pp. 164. (Institute of Physics: London and Bristol; Crane, Russak: New York, 1975.) £6.50.

THE majority of published books on authentication in the art world are concerned either with stylistic criteria or are purely anecdotal in character and do little to illustrate the significant contributions made by scientific methods of examination in this field, particularly during the past few decades. The author of this book, himself a scientist who has made noteable contributions to the development of thermoluminescence dating which has revolutionised the authentication of pottery, has set out to redress the balance and describe how scientific methods are applied to the detection of forgeries.

The book is divided into four main sections, the first of which provides a general introduction to the subject in which the author discusses the limitations of stylistic judgements; presents some early examples of the detection of forgeries; discusses different types of fake, the forger's technical approach and the errors and stylistic anachronisms which he is likely to perpetrate, and the role of surface examination by microscopy and ultra-violet fluorescence in uncovering forgeries. The other three sections are concerned with paintings, ceramics and metals, respectively. There is also an appendix devoted to X-ray fluorescence of Chinese blue-and-white porcelain, radiocarbon analysis, and lead isotope analysis of ancient objects.

These sections are concerned much more specifically with scientific techniques of examination covering many case histories and ending with fairly extensive bibliographies which deal adequately with case histories but could perhaps have benefited by the inclusion of at least a few more references to the fundamentals of the scientific techniques used for the benefit of readers who may wish to widen their background knowledge. For example it is rather surprising to find no reference to Zimmerman's development of the fine grain technique of thermoluminescence dating in the section on ceramics nor any reference to a basic metallurgical text in the section on metals. Also, in this age of high specialisation, the expert, when writing outside his own field, is not immune from mistakes and misconceptions, sometimes of an elementary nature. Thus in the section on metals, one discovers a statement (p125) which assumes that the production of tin bronze is associated with a significantly lower temperature technology than that of copper alone.

It is also inevitable that any publication concerning the authentication of valuable objects must be somewhat circumspect in its treatment of the material in order to avoid possible legal complications. Thus in its reliance on published examples in which the results of scientific examination are unequivocal, the book tends to give the impression that scientific techniques are rather more certain in the answers that they can provide than is actually the case. In general, however, and in spite of these limitations, the book is an excellent introduction to the scientific detection of forgeries and a good source of references to the literature of authentication and associated scientific studies. □

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Source of constructive thinking

Marcel Florkin

The Evolution of the Bioenergetic Processes. By E. Broda. Pp. vii+211. (Pergamon: Oxford and New York, 1975.) £8.30; $20.

In this much needed synthesis about the sources of free energy and their evolution in organisms, Professor Broda has collected and integrated a vast body of knowledge. He begins with a detailed exposition of current views on the origin of 'eobionts' and considers as most ancient those bacteria which are able to make ATP (the universal source of free energy in metabolism) anaerobically in a solution of organic compounds without direct participation of light. Such 'fermenters' (found among *Clostridia* and among methane formers, both gram positive) could only depend on the chemical energy of solutes. As the biomass increased, critical conditions arose—the production of compounds with high energy content by radiations, and so on, falling behind the rate of consumption of redox compounds useful in metabolism. At this point additional sources of free energy were acquired by photosynthetic bacteria through emergence of photosynthetic phosphorylation: in light, useful carbon compounds were regenerated from waste, and life could persist on the basis of antagonistic dark and light reactions.

Photo-organotrophs such as purple non-sulphur bacteria use as electron donors in the light, in anaerobic conditions, those organic electron donors which were used by fermenters in the dark. In addition to ATP, the expansion of life required, for the assimilation of CO_2, an external supply of electrons. Photolithotrophs such as *Thiorhodaceae* or purple sulphur bacteria and *Chlorobacteriaceae* or green sulphur bacteria) learned, through photochemical promotion, to use inorganic reductants as electron donors.

Photolithotrophs are removed from fermenters by one more step than photo-organotrophs. As photolithotrophic bacteria never produce oxygen (they do not extract electrons from water) the atmosphere remained anoxygenic, but sulphate (a mild oxidant) became plentiful in the biosphere. Oxygen increased in the atmosphere after the emergence of the ability of green plants (phytotrophs) to photolyse water and use it as reductant. Oxygen is toxic for anaerobes and it must be considered that a progressive oxygen tolerance was acquired by aerobic organisms who developed enzymes (oxygenases) through which oxygen was first exploited in biosynthesis. The author concludes that all respiring bacteria (relying on electron chains) have developed from photosynthetic bacteria and that the electron chains of respiratory phosphorylation were derived from the electron chains of photosynthetic phosphorylation.

It should be noted that non-photosynthetic lithotrophs (chemoautotrophs) as well as respiratory organotrophs, rely on oxidation in air to obtain ATP, resulting, in both, from respiratory phosphorylation, with the distinction that in the case of non-photosynthetic lithotrophs, the reductants are inorganic. The author also discusses the evolutionary positions of the variants of respiration (anaerobic and aerobic) in prokaryotes. He suggests that sulphate respirers (using sulphate instead of oxygen as the terminal electron acceptor in the electron flow mechanism characterising 'respirers') arose from some purple non-sulphur bacteria.

Bacteria and blue-green algae are prokaryotes; all other algae, higher plants, fungi, *Protozoa, Metazoa* are eukaryotes. Eukaryotes possess specific organelles (mitochondria, chloroplasts) housing the enzymatic machinary or respiration or photosynthesis. All eukaryotes are aerobic and are derived from eukaryotic protists (Metazoa, or higher animals, derived from Protozoa; Metaphyta, or higher plants, derived from green algae).

Professor Broda discusses the evolution of intracellular organelles in eukaryotes and the theories considering mitochondria and chloroplasts and endosymbiotes.

The book is completed by a discussion of the aspects of differentiation of tissues in the frame of oxygen need, that is, the further line of bioenergetic evolution among animals and plants. The author also considers the incidence of paleontological and geological evidence on the path of bioenergetic evolution deduced from the properties of extant prokaryotes and eukaryotes, and in the last chapter, retraces the history of atmospheric oxygen.

All students of evolution must be grateful to Professor Broda for providing such a lucid and documented discussion of this important topic. His book fills a gap in the literature on evolution and will certainly remain for a long time a source not only of bibliographic information, but also of constructive thinking. ☐

Photoreceptors

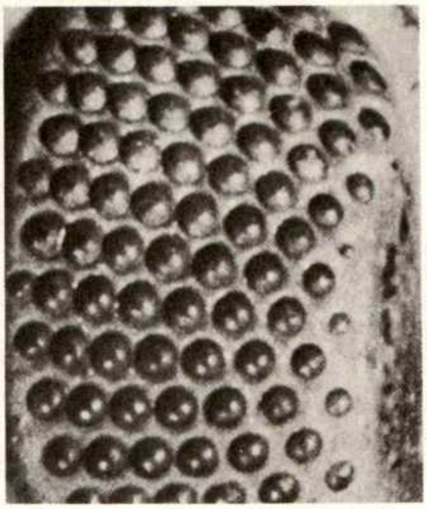
Aubrey Knowles

Photoprocesses, Photoreceptors and Evolution. By Jerome J. Wolken. (Academic: New York and London, November 1975.) $19.50; £10.15.

I WAS sadly disappointed that the promises of the title of this book, and its preface, were not fulfilled. The rapid progress of research into photosynthesis, vision and other fields of photobiology would make an overview of the relationship of the sun to life processes most timely. Unfortunately, Dr Wolken's approach is seriously biased by his own interest in invertebrate photoreceptors, resulting in a book that is badly out of balance—for example, the vision chapters occupy 110 pages, photosynthesis is confined to 27, and evolution, although appearing in several section headings, is barely mentioned in the text.

At best, the book presents a survey of visual photoreceptor structures in vertebrates and invertebrates, but most of the information, including the illustrations, appeared in Dr Wolken's earlier monographs. Beyond this, the text is a mixture of wide-eyed statements about the wonders of nature, and a mass of unrelated and often misleading facts, making a book that is not only difficult to read but that has to be read with the greatest wariness. In his preface, Dr Wolken says that the book is not intended for experts, but I find it hard to recommend it to anyone without a fair knowledge of both photobiology and evolution: there is much that is irrelevant, out-of-date, and—more serious—much that is omitted; for example, a Jablonski energy level diagram (carelessly copied from another author) is included without any explanation; a page is devoted to the geological time scale, but this is not mentioned again; 10 pages are given to a short account of bioluminescence that seems to have no place in the text, for in the author's own words, "The relationship of bioluminescence to photoreception . . . in evolutionary development is far from understood"; and so on.

The book is nicely produced, but I am curious about the two-inch wide margins; are these intended to raise the book into the 'coffee table' category, or are they for the reader's own additions? I had hoped to learn more about the bearing of light on evolution, but beyond token references to Darwin, Mendel and Karl Marx, the only photobiological system discussed in evolutionary terms is vision. Even in this restricted field, the fascinating story of the development of photoreceptors is barely mentioned, and we are given little more than a repetition of George Wald's 20-year-old theories on the evolution of visual pigments. There is no mention of the work of Bridges, Munz, McFarland and others over the past 10 years even though this has provided some hard facts about the relationship of visual pigments to environmental factors. In view of this poor treatment of evolution, and the sketchy information on primary photoprocesses, I suggest that the intending reader should ignore the first and third words in the title, and regard the book simply as a rather limited survey of photoreceptors. □



Part of compound eye of *Phacops rana*, a Devonian trilobite fossil

George Duncan

Photoreceptor Optics. Edited by A. W. Snyder and R. Menzel. Pp.x+523. (Springer: Berlin and New York, 1975.) DM 97; $41.80.

THIS book comprises a collection of papers that were presented at a colloquium in October 1974. Any text that is published within nine months of the meeting that conceived it is indeed remarkable and *Photoreceptor Optics* is doubly laudable because its chapters are so cross-referenced and deftly edited that we are made to feel that the authors were interested and indeed stimulated by the papers of their coparticipants.

The text is concerned with "how the optical properties of photoreceptors —their arrangement, orientation, shape, size, refractive index and membrane properties—influence their absorption of light and establish many of their specialised functions", and the initial section gives the most comprehensive treatment available of the role of wave guide optics in photoreceptor processes. The excellent theoretical chapter by A. W. Snyder is essential reading for all who debate the reasons for the intrinsic directionality of photoreceptors, or the importance of optical coupling between receptors.

After the mathematical rigour of the initial section, the second and third sections on photoreceptor membranes provides light relief, especially when introduced by a contribution by P. A. Liebman that manages to be both nutritious and easily digested. He provides a good review of the classical problem of determining the contents of photoreceptor cells by optical means, but he also gives us a glimpse of his very interesting present work on the axial diffusion of photopigment in cones. His findings go a long way to explain the difference in the distribution pattern of radiotracers in vertebrate rods and cones found by R. W. Young. He also focuses our attention on the small and hitherto unnoticed axial gradient of birefringence in rods, and on the basis of the observation, he spins a marvellous (and convincing) tale of the ageing of lipids in the rod–disc membrane.

Although the importance of pigment granule migration is acknowledged throughout this book, there is only one short paper on the possible photomechanical mechanisms at play, and that, by W. H. Miller, simply amplifies an earlier work by Miller and Cawthon. They suggest that microtubules mediate the movement of pigment granules in *Limulus* and they base their argument on the fact that the application of colchicine causes the same centripetal movement of shielding pigment as light does. It would be interesting in this respect for someone to repeat the experiments of W. A. Hagins who reported in a brief communication ten years ago that there was in fact no pigment migration when the illuminated photoreceptor (in this case cephalopod) was bathed with sodium-free medium.

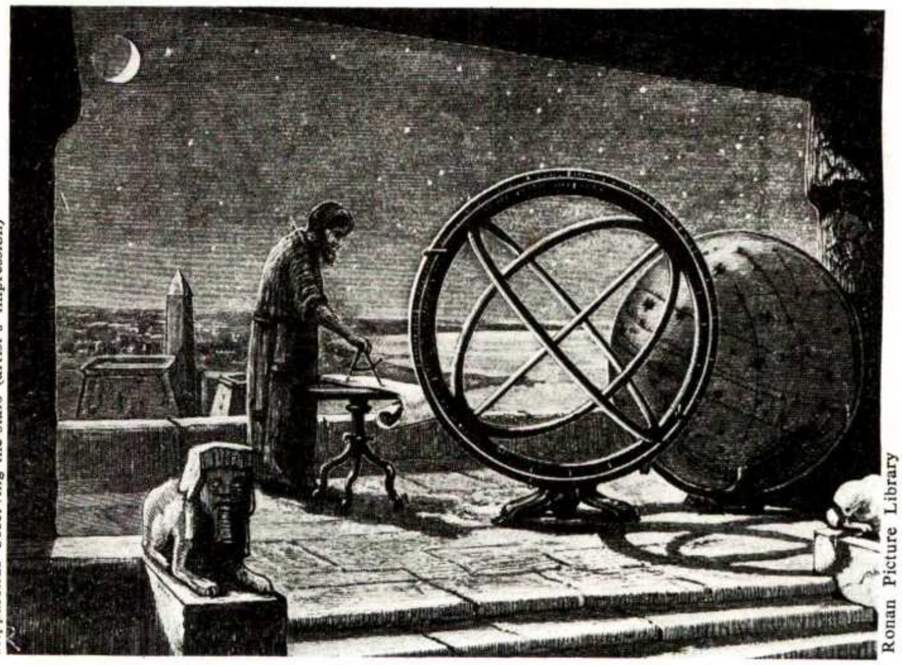
The great complexity of the arthropod photoreceptor system is highlighted by G. A. Horridge in the concluding section. From an analysis of intracellular microelectrode data, he found that there were two discrete sizes of quantum bumps in cells 1–6 of the fly's ommatidium. The miniature potentials elicited by 333-nm light are in fact larger than those produced by 500-nm light incident on the same cell, and so it seems that there are probably two transduction mechanisms within the same cell. It also seems likely that one pigment lies above the other and therefore orthodromic illumination and antidromic illumination may be differentially absorbed.

Although this text is within reach of the corporate rather than individual pocket, the conscientious researcher in the field of photoreceptor optics should ensure that he has ready access to a copy. □

Classic work on astronomy

G. J. Whitrow

A History of Ancient Mathematical Astronomy. Part 1: Pp. xxi+1–555. Part 2: Pp. 556–1,058. Part 3. Pp. 1,059–1,456. (Studies in the History of Mathematics and Physical Sciences, Volume 1.) By O. Neugebauer. (Springer: Berlin and New York, 1975.) DM 290; $118.90.



Hipparchus observing the stars (artist's impression)

Ronan Picture Library

PROFESSOR Otto Neugebauer, of Brown University, presents us with a magnificent work that will surely become a major classic in the historiography of ancient astronomy, a three-volume treatise devoted almost entirely to the mathematical astronomy of the Babylonians and of the Greeks.

He begins by solemnly warning his readers that "Many things are omitted here. The reader who wants to read about Archimedes taking a bath or about the silver nose of Tycho Brahe can find innumerable books which dwell on these important, biographical matters . . .". Instead, Neugebauer deals exclusively with "numerical, geometrical and graphical methods devised to control the mechanism of the planetary system". He omits any discussion of the theory of instruments (and also of Chinese and Mayan astronomy, neither of which had the slightest influence on the work of those about whom he writes).

For about 1,500 years (from the second century AD to the seventeenth century) mathematical astronomy in Europe and Islam was dominated by Ptolemy's *Almagest.* Book 1 of Neugebauer's *History* is therefore devoted to that tremendously influential work and its direct predecessors. He begins with two chapters on the plane and spherical trigonometry known to Ptolemy (about 150 AD), chords being used instead of halfchords, or sines (a later invention of the Hindus), and absence of knowledge of the fundamental formulae for right spherical triangles being compensated for by ingenious use of an important theorem attributable to Menelaus (98 AD). There follow three chapters on spherical astronomy before we plunge into Ptolemy's lunar theory (pp53–144) and his planetary theory (pp145–261). The remaining 80 or so pages of Book 1 are devoted to Apollonius and Hipparchus. The most important question of attribution confirmed by Neugebauer's authoritative analysis, although originally attributable to H. Vogt (*Astronomische Nachrichten,* 1925), is that the still widely held view that Ptolemy's star catalogue was largely based on Hipparchus's, a hypothesis accepted by the influential early nineteenth century historian of astronomy Delambre (because of his "preference for any theory which would detract from Ptolemy's merits"), cannot be sustained. This conclusion has been accepted by K. Lundmark, Owen Gingerich, and others.

Neugebauer is convinced that Hipparchus used arithmetical as well as geometrical methods. Just as the latter were the peculiar contribution of the Greeks, so the former, it now seems, (due to the researches this century by Kugler, Neugebauer, Van der Waerden, Aaboe and others) owed much to the Babylonians. Consequently, we can view Greek astronomy in what Neugebauer calls "an entirely new historical perspective", for we now realise that Babylonian arithmetical techniques greatly influenced both ancient Greek and also medieval astronomy. Book II (pp347–555) of Neugebauer's treatise is therefore devoted to Babylonian astronomy. A significant difference between the records of Babylonian and Greek astronomy available to us is that the former are authentic contemporary documents, dating from the last three centuries BC, even if some are copies of earlier tablets. Nevertheless, we have no information about the development of the completed system of Babylonian astronomy, unlike Greek astronomy. "In short", comments Neugebauer, "we probably know as much and as little about Babylonian astronomy as a Greek astronomer of the Hellenistic age knew". It is a mathematical astronomy that "operates without any model of a spherical universe, without circular motions and all the other concepts which seemed 'a priori necessary' for the investigation of celestial phenomena". It is entirely arithmetical.

Book III, only 12 pages long, is on Egyptian astronomy, the only important contribution of the Egyptians being the 'Egyptian year' of a fixed length of 365 days. It was the 'backbone' for the computation of astronomical tables down to the Renaissance.

Book IV (pp571–776) is concerned with pre-Ptolemaic Greek astronomy. I cannot resist quoting a short passage from page 572: "I see no need", Neugebauer writes, "for considering Greek philosophy as an early stage in the development of science . . . One need only read the gibberish of Proclus' introduction to his huge commentary on Book I of Euclid's *Elements* to get a vivid picture of what would have become of science in the hands of philosophers . . .".

Book V is devoted to astronomy during the Roman Imperial Period and Late Antiquity (that is, down to the seventh century AD). Book VI (pp1,061–1,456) contains Appendices, a Subject Index (including proper names), Bibliographical Abbreviations and so on, a short Greek glossary, and nearly 250 pages of diagrams and other figures, often as many as six on a page.

There is no doubt that here at last we have an up-to-date definitive textbook of ancient mathematical astronomy. I have so far noticed only one minor omission, and it may be deliberate. It concerns Thales, the sole reference to whom (p604) is to discredit his alleged prediction of the solar eclipse of 28 May 584 BC. Twenty years ago Professor A. Wasserstein contributed a paper to the *Journal of Hellenic Studies* (114–116, **LXXV,**

Star-studded journey

Paul Davies

The Dark Night Sky: A Personal Adventure in Cosmology. By Donald B. Clayton. Pp. xii+206. (Quadrangle/New York Times: New York, 1975.) $9.95.

I HAVE never read an astronomy book quite like this one. The author, who is an American astrophysicist at Rice University in Texas and an internationally respected scientist, has managed to weave a remarkable synthesis of personal narrative and descriptive astronomy into the same text. Not for Professor Clayton the dry old facts, figures and theories of conventional introductory astronomy books. Instead, reader discovers the motions of the Earth with the youthful Clayton delivering newspapers in Dallas. The mysteries of Stonehenge are unlocked as we accompany him in later years on a "sudden impulse" visit. Olbers' paradox unfolds from a detective story, as the author describes attempts to track down the family home of the eighteenth century astronomer de Cheseaux.

Much of this unusual book is taken up with the author's own research interest in stellar nucleosynthesis. Famous personalities like Fred Hoyle, William Fowler, Margaret and Geoffrey Burbidge appear as real people rather than mere names associated with pieces of work. To those, like myself, who worked in the early 1970s alongside Clayton and his friends at the Institute of Theoretical Astronomy in Cambridge, much of the account is nostalgic. In particular, the author's close friendship with Fred Hoyle (who has written a short foreword) comes through clearly—one whole chapter is devoted to their climbing exploits together in Scotland.

There is in fact much good science buried away in the text, but always interspersed with comment, narrative and a little Texas philosophy. The theory of relativity and some nuclear physics emerge from stories about Einstein and Rutheford, always embarked on by some personal incident. Emerging from the story is the unmistakable stamp of a romantic, helping to dispel the tired old idea of the cold, calculating scientist. The reader shares in the personal excitement which has changed the author's life. As we follow his daily experiences, science jumps out from everywhere: "Every aspect of nature" he explains "holds connections to the rest of the universe in my own mind . . . This sense of unity and knowledge has transformed my life from something mundane to something exciting".

Whether the reader wishes to learn astronomy by guided discovery, or to enjoy engaging stories of colourful personalities, Professor Clayton's book will provide an entirely welcome new perspective into the mysteries of astronomy and astronomers. ☐

(continued from previous page)

1955) on *Thales' Determination of the Diameters of the Sun and Moon*, followed soon afterwards by a further short note on the same subject, in which he argued that these determinations (ascribed to Thales by Diogenes Laertius) may have been obtained by angular measurement and not "as is generally supposed by time measurement". One other point concerns Neugebauer's conclusion (p643) that, in view of the highly inaccurate numerical values given by Aristarchus in his famous treatise *On the Sizes and Distances of the Sun and Moon*, this work should be regarded as "a purely mathematical exercise which has little to do with practical astronomy". I remember hearing a lecture by Professor Wasserstein ten or more years ago, in a colloquium at Imperial College, in which he made the same point.

The printing and presentation of all three volumes are up to the high standard we expect from Springer. Alas, not the least astronomical feature of this publication is its price. ☐

Flavour of excitement in modern astronomy

J. E. Beckman

Astronomy and Cosmology: a Modern Course. By Fred Hoyle. Pp. xiv+711 +23 plates. (Freeman: San Francisco, 1975.) $15.95.

ASTRONOMY has been a shining beacon in the subdued world of physical science during the last decade. Whereas research funding for most branches of physics and chemistry has been in the doldrums, money for astronomical research has been expanding in real terms. The opening up of new ranges of the spectrum by new techniques and by the use of satellites, has meant new discoveries. One needs only to run through a familiar list—radio galaxies, supernova remnants, quasars, pulsars and black holes—and to realise how many of these have become almost household terms, to understand why students have been turning towards astronomy and astrophysics as rapidly as they have turned away from some other branches of science. As a cultural subject, astronomy has further attractions for students and teachers. The history of the subject, the oldest recorded branch of natural science, subsumes a series of revolutions in the way man has viewed his place in the world. Even today the cosmological questions about the origin, the future and macrostructure of the universe exercise a unique fascination for both laymen and professionals.

Against this background Fred Hoyle has written a textbook aimed at first-year undergraduates. In US terminology it is a college rather than university text. A rapid glance through the book gives a most favourable impression. The author has made full use of the excellent illustrative facilities of his publisher, and their *Scientific American* style of didactic drawing. The book is well provided with photographs, mainly from the Hale Observatories, of nebulae, galaxies, and spectra. Above all the beautiful typography and layout go well with the leisurely presentation, in whose 700 pages the author has felt free to develop his favourite topics at considerable length.

One necessary reason for this spread is the deliberate abstinence from formal mathematics other than simple algebra and the use of logarithms, throughout the book, and the relegation of most algebraic development to chapter appendices. Although this had led to many elegant verbal explanations of topics as diverse as star formation, the quantum theory of spectra, and Kepler's laws, the result is to weaken the presentation. A perceptive student's feeling of inferiority before the mathematics will be increased; worse, a less able student will not realise the pervasive need for mathematical method in linking the concepts presented.

Nevertheless. the concepts are well presented and physical insight is demanded of anyone working through the interesting sets of problems, whose main virtue is to foster a feeling for orders of magnitude. The choice of material is a useful balance between classical introductory astronomy, and such modern topics as plate tectonics, and the physics of the 'big bang'. The author's occasional sallies into idiosyncrasy are carefully prefaced by "it is my belief" and like caveats.

I find the book excellent value at $15.95, and although not entirely suited to a British first-year undergraduate course, as a gift to educate and interest a sixteen- to eighteen-year-old it is almost ideal. It does convey the flavour of excitement in modern astronomy. ☐

Mysteries of the angiosperm

W. G. Chaloner

Palaeobiology of Angiosperm Origins: Problems of Mesozoic Seed-Plant Evolution. (Cambridge Earth Science Series.) By Norman F. Hughes. Pp. vii+242. (Cambridge University: Cambridge, London and New York, January 1976.) £7.80.

THE past decade has seen a renewed surge of interest in the evolutionary origin and diversification of the angiosperms. Four major books (Hutchinson 1969, Taktajan 1969, Sporne 1974 and Stebbins 1974) have dealt with the problem, placing varying degrees of emphasis on comparative studies of living forms, with a proportionate lack of emphasis on the fossil record. This approach takes its most extreme form in Stebbins' assertion that "the fossil record of angiosperms can be more misleading than enlightening as a means of interpreting the major trends of their evolution". Hughes offers an encouraging refutation of Stebbins' morbid view. His book is a palaeontologist's unrepentantly partisan survey of the fossil record of angiosperms, and of the reputedly relevant gymnosperms of the past 200 Myr. He has as little time for comparative studies of living forms as Stebbins has for fossils.

Two important facts emerge from his thorough and well-illustrated analysis. First, there is no acceptable evidence of angiosperms before the early Cretaceous. Second, all those characteristic attributes of angiosperms which have a sporting chance of surviving in a fossil (wood with vessels, dicot-type leaves, multiaperturate tectate pollen and seeds enclosed in a fruit) appear within the timespan of one geological stage, during or close to the Aptian (late early Cretaceous). A vital contribution here has been the palynological evidence, repeated synchronously at widely separated sites, showing the sequential change from monaperturate to multiaperturate pollen types.

Hughes places great emphasis on the data-handling aspect of the angiosperm evolution problem. He is rightly concerned that a question-begging nomenclature should not conceal the inadequacies of our fossil record. But in some instances this is carried to alarming lengths. He worries over the application of 'dicot' to Cretaceous woods or leaves, because the number of cotyledons is not directly evident in the fossils (he prefers the label 'dubiocot'). One wonders whether as a palaeontologist, he objects to attributing Cretaceous skeletal material to the mammals, since that designation is equally based on an attribute so singularly lacking in the fossil record?

The author offers us no snap answer to the mystery of angiosperm origins. But he has faith that the fossil record, properly documented, will eventually yield an answer. He hints that he believes the angiosperms to be polyphyletic, and that most of the major Mesozoic gymnosperm groups are under suspicion of duplicity. While we are awaiting that answer, this book is a most readable source of well-ordered factual information, and a strong goad to a renewed assault on one of the most rewardingly unsolved problems of biology. ☐

Jurassic-Cretaceous geology in Russia

John W. Neale

The Jurassic-Cretaceous Boundary and the Berriasian Stage in the Boreal Realm. Edited by V. N. Saks. Pp. vi+345+46 plates. (Israel Program for Scientific Translations: Jerusalem; Wiley: Chichester, September 1975.) £17.50.

ONE welcomes the translation of this impressive work which starts with a historical review of classification about the Jurassic–Cretaceous boundary, followed by detailed descriptions of the lithology, faunal succession, ethology and taphonomy of each relevant section in the Boreal Realm of the USSR. Western European developments are covered, although some up-dating is necessary in the light of recent publications, and correlation between the various areas is suggested. It is refreshing to find oneself in accord with this authoritative team of Russian workers in their reservations and strictures on the use of the term 'Ryazanian' and their preference for 'Berriasian'.

An extended review of the ammonites, belemnites, bivalves, brachiopods and foraminifera, read in conjunction with the 46 plates at the back, provides a fascinating insight into the Russian faunas. Although individual specialists may take issue with some of the generic or trivial assignments, this part provides an invaluable guide to current Soviet thought on the interpretation of these groups. Both here and elsewhere a considerable number of new fossil species has been described by the editor/author whom the Russians transliterate as 'Sachs', so that the use of this form is obligatory in the attribution of these taxa and is correctly used in the translation. It is a pity that the translators feel obliged to follow western precedent and use 'Saks' on the cover and elsewhere in the running text. That 'Sachs' and 'Saks' are one and the same is a potential source of confusion, particularly to the non-specialist.

Otherwise, the translation is good, the occasional infelicities only serving to suggest the flavour of the original. Incidentally, both original and translation show a curious anomaly—in table 24, in which the *Bojarkia mesezhinkowi* zone is placed in the Valanginian in contrast to its firm placing in the Berriasian elsewhere. Interesting chapters on palaeogeography (although the maps take no account of continental drift and are rather small in the translation) and on palaeogeographic zoning, close the book. This handsome, if somewhat expensive, volume is beautifully produced and besides being an exciting mine of information to dip into, will remain an indispensable work of reference for many years to come.

Ecology and Evolution of Communities
Martin L. Cody and Jared M. Diamond, editors
This book contains 18 major original papers, offering new models, methods and applications for analysing and interpreting a wide variety of empirical data. *Part I, The Evolution of Species Abundance and Diversity;* Richard Levins, Egbert G. Leigh, Jr, John W. MacArthur, Robert M. May, Michael L. Rosenzweig. *Part II, Competitive Strategies of Resource Allocation;* William M. Schaffer and Madhav D. Gadgil, Henry H. Hespenheide, Arthur M. Shapiro, Henry S. Horn. *Part III, Community Structure;* Martin L. Cody, James R. Karr and Frances C. James, Eric R. Pianka, James H. Brown, Jared M. Diamond, Ruth Patrick, Joseph H. Connell. *Part IV, Outlook;* G. Evelyn Hutchinson, Edward O. Wilson and Edwin O. Willis. *Belknap Press* **£17.70**

Anatomy of the Guinea Pig
Gale Cooper MD and Alan L. Schiller MD
This is a thorough description of guinea pig anatomy illustrated with over 400 drawings by Dr Cooper, covering: external anatomy; the skeletal, muscular, cardiovascular, lymphatic, central nervous, peripheral nervous, respiratory, digestive, urogenital and endocrine systems; the ear; the eye and orbital contents. *Commonwealth Fund* **£20.00**

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Larger-than-life characters

M. J. S. Rudwick

The Dawnseekers: The First History of American Paleontology. By R. W. Howard. Pp. xiii+314. (Harcourt Brace Jovanovich: New York and London, October 1975.) £3.95.

THIS is a popular history of palaeontological research in the US, from the first great vertebrate finds in the eighteenth century to the full professionalisation of the science in the twentieth century. The style is journalistic, with racy narrative and fictional dialogue. The subject matter should be ideal for a good popularisation of the history of science, for many of the personalities involved were as colourful and as larger-than-life as the extinct animals that they discovered and reconstructed. The author claims that "*The Dawnseekers* was not meant to be a definitive history", but even the most modest popularisation should utilise the more accessible results of modern historical scholarship. The best parts of *The Dawnseekers* are those that describe the epic feats of Cope, Marsh and others in the newly-opened American West; but it is sad that the opportunity of setting this work reliably in its historical context has been so completely missed.

The story is still framed in the terms set (or at least, propagated) by that old warhorse of the 1890s, Andrew D. White, as a conflict between crusading scientists in shining armour and a benighted army of clerics and other obscurantists defending the sanctity of Ussher's date of 4004 BC. This kind of interpretation is inexcusable in the 1970s, when several readable books based on accurate historical research are easily available. To mention only one, the omission of which is all the more surprising because it rightly emphasised the importance of American discoveries, John Greene's *Death of Adam* (1959) placed those discoveries firmly in their historical context as part of a continuing debate on the implications of the history of life for human self-understanding.

Science needs good popularisations, and popularised history of science is an excellent way of communicating the historical dynamics and humanity of the scientific enterprise. Unfortunately the simplistic journalism of *The Dawnseekers* will do little to advance the lay understanding of science. □

Preparing *Brontotherium gigas*, 1900

Courtesy American Museum Natural History

Geological clocks

A. Hallam

Growth Rhythms and the History of the Earth's Rotation. Edited by G. D. Rosenberg and S. K. Runcorn. Pp. xvi+559. (Wiley-Interscience: London and New York, September 1975.) £22.50.

IN the 12 years that have elapsed since Wells published his pioneer paper on corals as geological clocks a large amount of research has been conducted on growth rhythms in living and fossil organisms in relation to solar and lunar periodicities. This book, the outcome of a NATO conference held in Newcastle-upon-Tyne, summarises this work, and contains in addition a number of interesting papers by geophysicists and astronomers on the Earth's rotation. These range from an analysis of eclipses from ancient historical records to more theoretical studies of the dynamics of the Sun–Earth–Moon system. Greatest interest attaches to the study of rhythmicity in fossil growth layers as the best hope of obtaining hard data on diurnal, monthly and annual cycles in the distant geological past. Although it is impossible to summarise adequately in a brief review the results of so many diverse contributions, it is apparent that early efforts in the field greatly underestimated the complexities in growth patterns that emerge from more detailed analysis, and that stromatolites come a poor third to bivalves and corals as research material. Some rhythms perceived in living organisms relate more to varying environmental conditions than to astronomical periodicities, and misinterpretation of fossils is likely to arise unless one has access to large amounts of excellently preserved material with continuous growth records. There is also a need in the future for more objective measurements utilising, for instance, the optical densitometer. Nevertheless there still seems to be qualified support for a gradual slowing down of the earth's rotation throughout most of Phanerozoic time.

The editors are to be congratulated on a finely produced and splendid interdisciplinary effort. □

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Of crops and men

Joseph Hutchinson

Crops and Man. (Foundations for Modern Crop Science Series.) By Jack R. Harlan. Pp. xi+295. (American Society of Agronomy and Crop Society of America: Madison, Wisconsin, 1975.) $11.25.

THIS is a good book. The expansion of studies of crop plant evolution in recent years has been so great that to produce a survey of the field in one volume is a daunting proposition. de Candolle and Vavilov provided respectively the roots and trunk of a tree that has now grown a crown almost too great to be encompassed by one man. Nevertheless, Jack Harlan has set out to do this. In his Preface he says: "The time has come for a third round of summary on what is known about crop origins".

Harlan has not only succeeded in accomplishing such a summary; he has contributed a most timely addition that substantially enlarges the field. de Candolle's interests were primarily botanical and geographical. Vavilov's were botanical, geographical and genetic. Harlan's are botanical, geographical, genetic and human. Although de Candolle was well aware of the human influences in the phenomena he studied, it was natural that for a long time the study of the origins of crops and of agricultural practice should be pursued separately. Their natural interrelation has been apparent in the past decade, and there are now numerous examples of the way in which biological and archaeological evidence together contribute to the elucidation of crop plant history. Harlan has taken these disciplines and fused them into a comprehensive account of the social as well as the biological changes that went into the evolutionary progression from hunting and gathering to a full agricultural economy.

The sponsors of the series of which this is the first volume, have sought to meet the needs of "upper level undergraduate college students". For students reading subjects in applied biology, archaeology, anthropology and geography this will be mandatory reading for a long time. It will also be invaluable for teachers who are developing 'A'-level Environmental Studies in schools.

This subject is my absorbing interest, and it would be surprising if I agreed with all of Harlan's views. I would add a South Asian Region to his list of original crop regions, and would assign to it such crops as the Indian pulses (including pigeon pea), *Gossypium arboreum*, and some tetraploid and hexaploid wheats. I think he has overdrawn the protein shortage, and I do not think one can accept his statement (p253) that "it was not until World War II that significant increases in per hectare yields were achieved". There were at least two earlier periods in British agricultural history during which substantial advances in yield were made. But these are matters for debate, and it is a measure of the stimulus of the book that one constantly made a mental note of a new or unrealised aspect of the subject—that the hunter–gatherer lived in a golden age, for example—or of an interpretation one did not accept and would like to discuss with the author.

The book begins with a discussion of the way people lived before they farmed, how they began to farm (leaving open the question why they should want to), and our present ideas of the areas in which farming began. Then there are chapters on crops and weeds—which is which and why— and on the classification of cultivated plants. Genetic and evolutionary topics follow. Five chapters are devoted to regional studies, and finally there is one on recent spread much of which can be documented historically, and one of interesting and well controlled speculation on future prospects and possibilities.

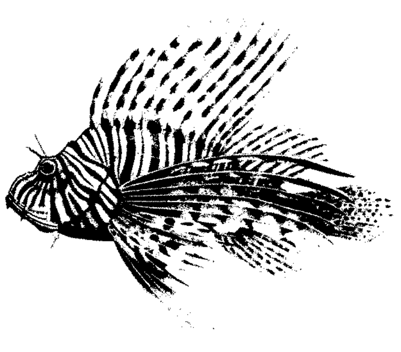
I enjoyed it. □

Air pollution

C. P. Whittingham

Responses of Plants to Air Pollution. (Physiological Ecology: a Series of Monographs, Texts and Treatises.) Edited by J. B. Mudd and T.T. Kozlowski. Pp. xii+383. (Academic: New York and London, November 1975.) $29.50; £15.35.

THE study of the response of plants to aerial pollutants is relatively new. In our present state of ignorance it is important to differentiate between proven facts and generalisations which have become established only by frequent repetition. There is a need to pose pertinent questions which require experimental investigation and to formulate primary problems in analytical terms. Unfortunately this book does not attempt these tasks. It consists largely of literature reviews of current work and since all the authors are American most of the book relates to conditions in America and to work undertaken there.



Scorpion fish (*Pterois volitans*). Taken from *An Age of Fishes: The Development of the Most Successful Vertebrate.* By Joy O. I. Spoczynska. Illustrated by Melchior Spoczynski. Pp. 145. (David and Charles: Newton Abbott, London and Vancouver, February 1976.) £3.95.

Separate chapters discuss the influence of sulphur dioxide, ozone, fluorides and peroxyacyl nitrates, oxides of nitrogen, and particulates. Some chapters include more references than others and are correspondingly more valuable, but essentially all are concerned with reviewing the literature rather than discussing principles. Subsequent chapters deal with the response to mixtures of pollutants and the interaction of air pollutants with vegetation. There is little reference to the importance of the physiological status of the plant—for example, the influence of water stress or mineral deficiency in relation to plant response to pollutants —and there is only a limited reference to the importance of aspect in relation to pollution damage. One chapter is concerned with the interaction of pollutants with vegetation canopies; unfortunately there is little discussion relating the spread of pollutants to physical processes. Other chapters are concerned with the specific effects of pollutants in forests and in relation to agriculture. There is also a chapter on the influence of pollutants on plant structure and on the growth of lichens and bryophytes. In many chapters reference is made to the biochemical action of specific pollutants and perhaps it is premature to seek an account of the general interaction of pollutants with plant metabolism.

The book will be useful to those who require an introduction to the literature of plant response to pollutants. It does not provide a critical view of our present state of knowledge of the subject. It makes general statements without quoting evidence such as "yields of virtually all important crop plants have been greatly depressed by air pollution"; with certain exceptions it fails to emphasise the important facts which still need to be established in the face of the possible need to define air quality standards for future legislation. □

Ecology of rivers . . .

D. H. Mills

River Ecology. (Studies in Ecology, Vol. 2.) Edited by B. A. Whitton. Pp. x+725. (Blackwell Scientific: Oxford and London, 1975. Distributed in US by University of California Press.) £18.

River Ecology is the second volume in this internationally-based series concerned with ecology which aims at the production of scholarly integrations of established topics and critical assessments of newly-opened fields. This volume offers a comprehensive treatment of river ecology, and its prime aim is to provide a fundamental framework for the subject. Such an approach will be of value both to the more advanced student and to the research worker and river management officer who needs some guidance to the mass of literature which is now appearing about rivers. The 28 contributions to this book go a long way to fulfilling these aims and have certainly guaranteed that it is one of the few, although not the cheapest, books in English which covers so many aspects of river ecology.

Unavoidably the contributions vary in their quality and length, and their uneven size does not necessarily correspond to the importance of their subject. Some are disappointingly short, whereas others are surprisingly drawn-out. For example, only 31 pages are devoted to fish, whereas a similar number of pages deal with lotic periphyton. No-one would question the great importance of zonation in river ecology but the 63 pages devoted to this subject are more than twice the number of pages given to any of the other contributions except two.

In a book of this sort, where the size of the author's contribution is necessarily limited, it is not difficult to find omissions. The most glaring omissions occur in the chapter on Ecological Aspects of River Impoundment. This chapter tends to dwell almost entirely on the effects within the impoundment, and the downstream effects of impoundment on rivers are described in two pages. Only ten lines are devoted to the effects of hydroelectric installations on river flows and fish movement, and no mention is made of the effects of water transfer, either directly from river to river or indirectly through a regulating reservoir. This is a subject of paramount importance in the water resources field in this country at the moment, and its omission is to be deplored.

In the chapter on River Zonation and Classification a number of photographs are included which depict the various zones, named after the dominant fish species present, in a number of river systems. A number of these do not correspond with the physical appearance of these same zones in other river systems. For instance, the example given of the minnow reach or grayling zone (Fig. 14.2a) is nothing like the grayling zone on the River Tweed or the River Clyde; and the minnow reach in some Welsh mountain streams would correspond almost to the head stream depicted in Fig. 14.1a. The author refers to the River Wye, at Newbridge-on-Wye, (Fig. 14.2b, and the front cover of dust jacket) as being an upper salmonid zone although grayling, dace and chub are abundant in this stretch of the river. I would not refer to the Wye being an upper salmonid zone until one reached a point upstream of Llangurig.

The book ends with an Appendix which gives a guide to Further Literature on Particular Rivers. There are a number of glaring omissions, however, which include the Teifi and Towy in Wales, the Bran, Conon, Don, Forss, Meig and Tay in Scotland and the Fraser, Margaree, Miramichi and Pollet in Canada.

This is, however, a most valuable work on river ecology and I recommend it being purchased by all appropriate university departments and research institutions, and by more affluent research workers. □

. . . and animal populations

M. E. Solomon

Animal Population Ecology. By J. P. Dempster. Pp. x+155. (Academic: London and New York, August 1975.) £3.80; $10.00.

THIS thin, hard-backed volume provides good, if mostly brief, coverage of its subject, dealing with 'basic concepts', competition, social behaviour, qualitative changes, natural enemies, weather, and examples of field studies, and concluding with population theories and applied population ecology. The comparative account of selected field studies is a major and valuable feature. For each of them, life tables are presented and analysed by the Varley and Gradwell method for key factors, to diagnose where, if at all, in the life cycle there occurs a mortality that is the chief component of the fluctuations in numbers over successive generations. The book also attempts to diagnose which process is responsible for the limitation of numbers. In four of the six examples, this density dependent process seems to be intraspecific competition, for food in the cinnabar moth, wood pigeon, and pine looper in its outbreak areas, for settlement sites in the edible cockle, or for territories in the tawny owl. Three other field studies, on larch tortrix, red grouse and rock ptarmigan, are considered in connection with the problem of how periodic cycles of these species are engendered.

The author's grasp of the theory of population dynamics seems to falter in places. The intrinsic rate of increase (r) equals births minus deaths (p. 4) only in the infinitesimal interval of the calculus equation describing increase; over a finite period the relationship is not so simple. It is not true that k-factor analysis necessarily enables us to recognise "any density dependent relationships which exist" (p. 15). The statement (p. 140) that "the maximum sustainable yield......in the logistic model......is $K/2$", can hardly be correct, since in this context yield is a rate, whereas $K/2$ is a quantity.

The exposition is occasionally faulty. On page 17 the explanation of k-values is confused by the introduction of an index of mortality that is insufficiently explained by the footnote. In Table XVII the values are divided by 10^6 and so on, not multiplied as indicated above the columns. The phrase "sufficiently density-dependent to prevent extinction" (p. 105) may lead students to suppose that a density-dependent influence at low density can avert population extinction in some positive way (whereas the most it can do is to cease its adverse action). On the verbal level, it is disconcerting to find Pimentel, one of the theorists whose views are examined, misspelled in both text and reference list. 'Epidemic' should not be treated as a synonym of 'epizootic' (p. 43). Hassell's term is 'aggregative', not 'aggregate' response (p. 49).

In spite of these blemishes, however, this is a valuable book that should appeal to university students and research workers. It has an index and an up-to-date reference list of some 200 items. Its scope overlaps that of the earlier book by Varley, Gradwell and Hassell (*Insect Population Ecology*, Blackwell, Oxford, 1973), which goes more deeply into population dynamics and the relevant methods of analysis, but is confined to insects. □

On trial

Griffith Edwards

Brain, Behaviour and Drugs. By David M. Warburton. Pp. x+280. (Wiley: London and New York, December, 1975.) £6.95; $15.30.

TEXTBOOKS on psychopharmacology are these days appearing thick and fast and there might be a case for a 'Which' report. The present book is certainly a good buy, but as in every consumer's report there are a few things to niggle about. The particular attraction of this subject is that it enables authors to put on it an individual stamp, and here the stamp is characterised by authority and enthusiasm.

Dr Warburton first discusses the chemistry of synaptic transmission, and the effect of drugs on transmission. An interesting chapter then focuses on control of homeostatic motivation, with particular reference to water balance and food intake. A chapter on the biochemical basis of mood competently covers essential ground, and the section on control of attention gives a careful account of a difficult area. There is a good section on schizophrenia but affective illness does not get similar treatment, and intelligence and learning are also covered.

It is in the later sections that there is occasion for a few criticisms. The review on the influence of alcohol on the EEG is not up to date. The section on the biochemical basis of drug dependence, although basically very well handled, shows some flaws. For instance, the statistic is quoted that 22.5% of the psychiatric admissions in Brazil, India, Morocco and Nigeria were between 1957 and 1964 as a result of an acute cannabis reaction—a statement so bizarre as usefully to be put before students to test their critical faculties, but not to be coldly retailed as fact. To say that personality changes consequent on cannabis use "seem to be irreversible", runs some leagues ahead of the evidence, and the cannabis literature is in general not sufficiently well treated.

The chapter on anxiety and stress is also a little bit disappointing. The concept of anxiety and the concept of stress could both have done with greater eludicidation: the student might expect more about the measurement of psychophysiological correlates of anxiety. The discussion on the clinical application of anti-anxiety drugs on human beings errs on the side of optimism—not every psychiatrist would agree that "the debilitating effects of pathological anxiety have been reduced to a remarkable extent by a number of psychoactive drugs."

The usual difficulty with the teaching text on this subject lies in effectively making the linkage between the psycho- and the -pharmacology. This book succeeds more than most. The real way to test such a new product must of course be to try it out live on a panel of consumers, and here that means seeing how the book actually goes as a teaching text. This book certainly deserves that kind of trial. □

Neural organisation

H. F. Bradford

The Synaptic Organisation of the Brain: an Introduction. By Gordon M. Shepherd. Pp.xi+364. (Oxford University: London and New York, 1975.) £3.80.

WE have not yet reached the position where a definitive account of the synaptic organisation of the brain can be given, but Professor Sheperd provides us with a useful current appraisal of our degree of success in the drive towards that objective. He begins with a brief introduction to the morphology of synaptic and related structures and of neural connexions, and proceeds to expound the biophysical mechanisms which probably underlie resting transmembrane potentials and their displaced versions, the action and synaptic potentials. This is done in an explanatory way rather than with a vigorous mathematical approach. This leads on to an analysis of dendritic organisation in relation to passive spread of potential change due to synaptic activity, and the work of Wilfred Rall in this area. The second and most dominant part of the book deals with neural organisation in the ventral horn of the spinal cord, the olfactory bulb and cortex, the retina, the cerebellum, the thalamus, the hippocampus and the neocortex. The text is based on an advanced seminar course given by the author in recent years at the Institute of Neurological Sciences at the University of Pennsylvania, and its aim is to isolate and propose principles of synaptic organisation in these regions for the consideration of advanced students and research workers in neurophysiology. Neuropharmacological and developmental aspects of the subject are purposely restricted to keep the length of the book within reasonable bounds.

I found the flow and content of the book interesting and engaging as well as informative, and it is well linked to the literature of the subject with copious references (a large proportion dating from 1970 onwards). The absence of the more recent developments in our knowledge of catecholamine and indoleamine-operated pathways in the mammalian brain does detract from the success of the book as a description of synaptic organisation, but one appreciates the reasons for their absence. The accounts of the neurophysiological mechanisms of the various brain regions are clearly explained and well illustrated, and the rather conversational style of the text, together with the author's determination to present complex mechanisms as interplay of simpler more universal processes, adds to the ease of its reading. Each regional account deals with the range of neurone types present and the major types of synaptic connections existing between them. This is followed by an account of organisation in terms of circuit diagrams and the special features of that region in this respect. This is accompanied by a discussion of specialised dendritic properties crucial to the synaptic interactions. There are a large number of books in this area appearing at present, but this should prove to be among the most popular. By modern standards its price is attractive for its size and content, and in this respect too it should compete successfully on the bookstalls.

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Tranquillisers no answer to schizophrenia

Malcolm Lader

Drugs, Madness and the Brain. By Solomon H. Snyder. Pp. 287. (Hart-David, MacGibbon: London, 1975.) £6.

It would be a macabre task to draw up a league table of the most terrible diseases which can afflict mankind. In such a list, schizophrenia would be very near the top as this illness disrupts and destroys normal mental functioning. It is common, one in a hundred adults succumbing. The outcome is uncertain, some recovering unscarred, others with residual symptoms and strange behaviour, and an appreciable unfortunate group who occupy the majority of the 'chronic beds' in health services throughout the world. These patients exist in institutions ranging from true mental hospitals, with rehabilitation and return to the community as the aim, through lunatic asylums, where the patients are cared for to a greater or lesser extent, to 'bins', a pejorative but accurate slang term.

New hope dawned for schizophrenic patients, their families and medical attendants with the advent of the major tranquillisers 20 years ago, coinciding with more liberal attitudes towards the mentally ill. The numbers of patients in hospital declined although the number of admissions rose.

In this book, Solomon Snyder, a psychiatrist-*cum*-pharmacologist, sets out for the layman recent research into the biology and treatment of schizophrenia. In part I he outlines concepts of insanity, the treatments for schizophrenia and the effects of the psychedelic drugs like mescaline and lysergic acid diethylamide (LSD) pointing out that the psychoses which these drugs induce differ in several crucial respects from schizophrenia. Part II provides a superb non-technical description of the many mental aberrations and behavioural peculiarities of the schizophrenic patient. Finally, Snyder describes the psychosis induced by repeated high doses of amphetamine and shows how similar the mental state is to that of paranoid schizophrenia.

One tantalising question remains unanswered. What is the clinical type of action of the major tranquillisers such as chlorpromazine? Are these drugs anti-psychotic, that is, lessening psychotic symptoms whatever the causation, or are they truly anti-schizophrenic, influencing the schizophrenic disease process itself? Although Snyder heads one chapter "Anti-schizophrenic Drugs", he is at pains to point out the contradictions implicit in this rubric. For example, schizophrenic patients are not all cured and some sceptics have suggested that the major tranquillisers are merely 'chemical strait-jackets'.

The most relevant pharmacological effect of the major tranquillisers has recently become clearer. All these drugs have the common property of attenuating the action of the neurotransmitter dopamine by competitive, postsynaptic blockade. This explains extrapyramidal side-effects like Parkinsonism and some hormonal effects of the drug. It is tempting to postulate that schizophrenia involves dopaminergic overactivity in the brain but Snyder points out the dangers in such a facile extrapolation. This area of pharmacology is growing rapidly and should uncover the biochemical and physiological mechanisms associated with psychotic behaviour in general and (dare one hope) schizophrenia in particular.

The book is well written in non-technical language. It is entertaining, vigorous, optimistic in outlook but balanced in opinion. It can be heartily recommended to any intelligent layman who wants to keep abreast of research development in a major area of health concern. It should also be read by medical and scientific professionals from medical and psychology students to researchers involved in the field. And our administrators and politicians involved in the provision of health care might also take time to learn about schizophrenia—this dreadful life-long mental condition which afflicts so many thousands of patients vegetating in institutions or existing precariously in the community. Major tranquillisers are still not a substitute for adequate health services. □

Pervasive role for acetylcholine

T. J. Crow

Central Cholinergic Systems and Behaviour. By P. V. De Feudis. Pp. x+422. (Academic: London and New York, 1975.) £6; $15.75.

At a conservative estimate, established chemical transmitters in the central nervous system must now number at least seven, even excluding polypeptides which act as hypophyseal releasing factors, the recently discovered endogenous morphine-like compound, and the elusive substance P. Why so many substances should be needed to carry messages from nerve cell to nerve cell is a major unsolved question. The classical concept of postsynaptic excitations and inhibitions suggests a requirement for two, and the present surfeit might be thought to support the view that major varieties of action at the post synaptic cell have yet to be described.

Among these agents acetylcholine has a particular place, as the first such substance convincingly to be demonstrated to have a neurohumoral role, and by reason of the detailed knowledge we have of its release and action in the periphery. Yet its functions in the brain might be thought recently to have been neglected by comparison, for example, with the monoamines. This monograph by de Feudis contributes toward redressing the balance and reviews in detail the information which suggests that acetylcholine undoubtedly has a pervasive role. Although much of the subject matter concerns experimental findings at the electrophysiological and cellular level, the book is organised in terms of functional systems and behaviour. The author does not flinch from plunging the reader into a first chapter on cholinergic mechanisms and consciousness, and follows this with sections on motor systems, and the role of acetylcholine in autonomic and endocrine control, and in motivated behaviours, emotion, learning and memory. The reference in the title to behaviour is therefore justified and the book will be of particular interest to physiological psychologists. De Feudis has been unusually successful in his attempt to bring together a variety of relevant studies at the biochemical and cellular level and at the level of gross structure and function.

A text organised around a single neurotransmitter substance invites questions concerning the lead which information concerning this substance might give us to the functional organisation of neurotransmitter mechanisms in general. When does a pathway need to use acetylcholine as a neurotransmitter? Anatomical precision is crucial here, and as de Feudis makes clear, in spite of the development of the cholinesterase technique, there are many limitations in our present knowledge of the anatomical identity of cholinergic neurones. □

Adrenergic transmission

A. D. Smith

Adrenergic Neurones: Their Organisation, Function and Development in the Peripheral Nervous System. By Geoffrey Burnstock and Marcello Costa. Pp. xi+225. (Chapman and Hall: London, August 1975.) £8.50.

THIS comprehensive little book presents a summary of our knowledge of the peripheral adrenergic nervous system. It serves as an excellent entry into the literature, for the authors have quoted nearly 1,200 references which occupy 80 pages. Since only 136 pages remain for the text, it is inevitable that much of the writing is very descriptive. This is particularly so in the first two-thirds of the book, which gives a rather superficial account of the general organisation and structure of adrenergic nerves, the biosynthesis and metabolism of noradrenaline, and the basic features of neurotransmission. The remainder of the text, starting with a discussion about adrenergic transmission, continuing with a chapter reviewing the growth and degeneration of adrenergic neurones and ending with a general discussion, is much more original and contains some important new ideas.

It is hardly surprising that, in their wish to be both brief and comprehensive, the authors have occasionally oversimplified and have tended to make dogmatic statements. The reader is likely to be rather confused by the account of ganglionic transmission where it is stated that inhibitory muscarinic cholinoceptors occur on adrenergic neurones (p14) although elsewhere the muscarinic inhibitory effect is said to be mediated by interneurones that release a catecholamine. No mention is made of the excitatory muscarinic cholinoceptors in ganglia; perhaps it would have been wiser to admit that the events in the ganglion are complex and not fully understood. Likewise, it would have been better to say that we know nothing about the electrogenesis involved in the muscarinic action of acetylcholine on nerve terminals, which leads to inhibition of the release of noradrenaline evoked by orthodromic stimulation, rather than to imply that it is due to hyperpolarisation of the membrane. I assume that the authors supposed that hyperpolarisation of the membrane in the varicosities might block the invasion of the varicosities by the action potential; however, if the action potential is in fact conducted in the varicosities, then hyperpolarisation would increase its amplitude and so increase the amount of transmitter released.

The authors claim that the re-use of synaptic vesicles in nerve endings is not compatible with the idea that noradrenaline is released, together with dopamine-β-hydroxylase, from the vesicles by exocytosis (p34). I can see no inconsistency since after a vesicle, that has newly arrived in the terminal following transport from the cell body, has released its noradrenaline and soluble dopamine-β-hydroxylase, the vesicle membrane can probably be retrieved from the plasma membrane to form a new type of vesicle. The new vesicle is perfectly functional since it contains the (non-releasable) membrane-bound dopamine-β-hydroxylase and can store noradrenaline by means of the pump in its membrane. Such a re-use of the vesicle membrane provides an answer to the authors' problem that, if each vesicle is only used once, then the supply of vesicles by axoplasmic transport from the cell body to the terminal is apparently not adequate to maintain transmission (p91).

"Freckie (the otter) is displeased". Taken from *The River People.* By Philip Wayre. Pp. 180. (Collins and Harvill: London, 1976.)

One of the best features of the book is the discussion of neuroeffector transmission. Here the authors illustrate the key importance of the size of the gap between the terminal varicosity of a neurone and the effector cell. In some tissues (for example, vas deferens, nictitating membrane, sphincter papillae) the gap is about 20 nm, whereas in blood vessels the minimum gap is larger and is also more variable (80–400 nm). Functionally, this range of distances means that those tissues in which the gap is small respond to nerve stimulation more quickly and at lower frequencies of stimulation than do tissues in which the gap is greater. Many of the differences found by pharmacologists in the responses of the various adrenergically innervated tissues to drugs can now be accounted for in terms of the size of the neuromuscular gap. In tissues in which the gap is fairly wide, the peak concentration of noradrenaline at the receptors following nerve stimulation may be close to the threshold concentration. In these tissues, decreasing the amount of transmitter released, either by depleting the vesicle stores (with reserpine) or by interference with the release process (with bretylium), is likely to inhibit neurotransmission. In contrast, in tissues in which the gap is small these two drugs have little effect on transmission because the peak concentration of noradrenaline at the receptor may normally be more than 100 times the threshold concentration. Such a high peak concentration following nerve stimulation could also account for the lack of effect of α-blocking agents on neurotransmission in the vas deferens since the concentration of blocking drug would not be high enough to compete with the transmitter. Finally, the ability of drugs like cocaine to potentiate neurotransmission in some tissues but not in others is most easily explained by the different size of the neuromuscular gap.

The lack of uniformity in the organisation of adrenergic neuroeffector systems has led the authors to propose a fascinating new hypothesis that the terminal network of an adrenergic neurone not only permits direct control of the smooth muscle by the nervous system but also acts in those tissues that are densely innervated, to protect the tissue from the influence of circulating catecholamines. This protective function is the result of the ability of the adrenergic neurone to sequester catecholamines that are in the extracellular space; the denser the innervation, the more effectively are catecholamines removed. The authors point out that densely innervated organs such as the vas deferens and iris perform functions that are quite unrelated to those of the adrenal medulla. This hypothesis represents the first serious challenge to the classical views of Cannon, who saw the sympathetic nerves and the adrenal medulla as, functionally, one physiological system. This book is thus doubly welcome for it not only serves as a useful and comprehensive introduction to the field but also provokes physiologists and pharmacologists to take a fresh look at adrenergic neurotransmission. □

Brain power for astronomy

W. H. McCrea

The Place of Astronomy in the Ancient World. (A Joint Symposium of the Royal Society and the British Academy.) Edited by F. R. Hodson. Pp. 276+25 Plates. (Oxford University: London, 1975) (Published for the British Academy.) £13.

'ASTRONOMY in ancient literate societies' (Part I) starts with a useful introduction to needed astronomical concepts and closes with an account of two uses of ancient astronomy, both contributions by R. R. Newton. These ensure maximum comprehensibility of the work and appreciation of some of its objects, the two chosen by Newton being the study of the non-gravitational acceleration of the Earth and Moon, and the study of chronology, particularly from ancient eclipse records. The intervening contributions are on Babylonian mathematical astronomy, Babylonian observational astronomy, ancient Egyptian astronomy, astronomy in ancient and medieval China, and Mayan astronomy in central America. For most readers the striking general features must surely be the widespread character of astronomy in the ancient world and the inference that scientific astronomy in Greece, India, Islam and western countries was so much derived from Assyrian and Babylonian astronomy. Indeed, there is no chapter on Greek astronomy as such; presumably the characteristic Greek contribution is to be regarded as one of interpretation rather than discovery.

Part II is on 'Ancient astronomy: unwritten evidence' with contributions on 'Neolithic science and technology', 'Polynesian and Micronesian astronomy', 'Prehistoric monuments in western Europe', 'Astronomical alinements in Britain, Egypt and Peru', 'Archeological tests on supposed prehistoric astronomical sites in Scotland'. These deal with the most recent sort of study of ancient astronomy and that which has attracted most popular attention. There follows a chapter by H. H. Lamb on 'Climate, vegetation and forest limits in early civilised times' and one by D. G. Kendall on 'Hunting quanta'. These two are highly relevant in topic and they show the extraordinary range of science that has to be brought to bear on the problems concerned. The investigation as to whether the dimensions of megalithic sites reveal the use of a quantum (5.44 feet, 1.66 m) demands novel mathematical statistics of subtle ingenuity (but it seems not to have been tested by re-discovering the foot or the metre!). The book includes brief reports of some informed discussion at the meeting.

Within the allotted space, the book provides surely the best possible accounts of its topics. It is a feast of learning and scholarship, strongly spiced with wit and wisdom. It is thought-provoking in innumerable ways; some of these are noted in the concluding remarks by D. G. King-Hele and by S. Piggott from the sides of the physical scientists and the archaeologists. Naturally one of the most obvious features of some of the unwritten evidence is its uncertainty. On the whole, readers may be inclined to agree with King-Hele that some features of prehistoric sites did indeed mark extreme rising and setting points of the Sun and Moon, and the like, whereas a connection with eclipse prediction seems less likely. As regards the alinements, E. W. MacKie in his contribution remarks explicitly on the difficulties of the subject, but he then asserts: "The plausibility or otherwise of these alinements is something all can assess by visiting the sites". Actually a party of Cambridge undergraduates did subsequently visit some of the sites and came away rather dubious about the alinements (M. E. Bailey *et al.*, *Nature*, **253**, 431, 1975). But MacKie's assertion is surprising because the problem tackled by Kendall is the most straightforward in the subject and yet all his powerful statistical apparatus is unable to produce a definite conclusion from the available evidence. All the other inferences about the sites seem to be *a fortiori* quite indefinite—but fascinating and deserving of the most thorough-going investigation.

The brain power of even the most ancient of the peoples concerned—as King-Hele says—cannot have been appreciably different from that of our contemporaries. This raises all manner of questions about the progress of knowledge and understanding of the astronomical world. Our knowledge is more than that of the ancients, but is our ignorance as great as theirs? Does almost constant brain power produce accelerated growth of technique and accompanying increase in knowledge? Or do developments occur in fits and starts with peaks of knowledge and understanding and great gaps between the peaks? Is our knowledge placed on record in such a way that people 3,000 years hence will be aware of what we know much better than we are of what people knew 3,000 years ago? This book will provide material for endless debates. □

Scientific Research in British Universities and Colleges 1974-75

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Worm's eye view

Neil A. Croll

The Biology of Free-Living Nematodes. By Warwick L. Nicholas. Pp. viii+219. (Clarendon: Oxford; Oxford University: London, August 1975.) £8.25.

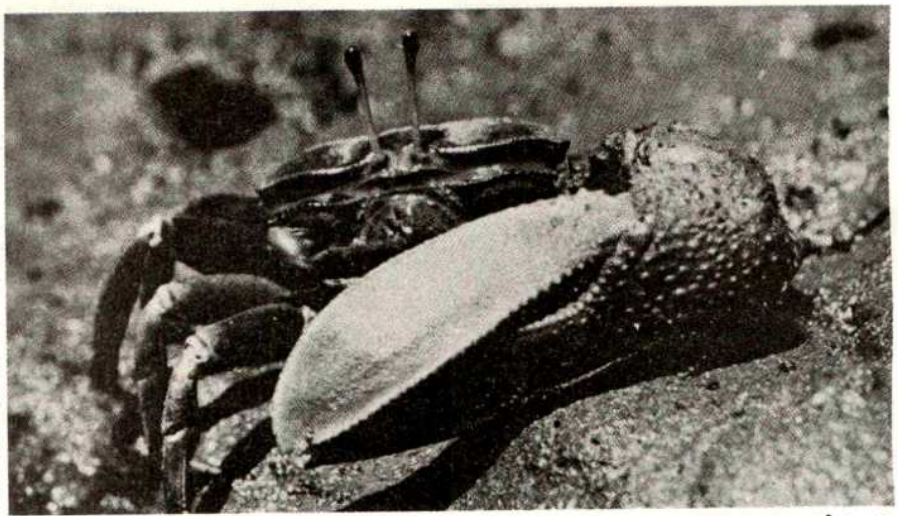
THE study of nematodes, perhaps more than with any other group of animals, is divided by the artificial barriers of professional groupings, journals and institutions. Too little communication exists between 'helminthologists', 'plant nematologists' and 'invertebrate biologists'. Dr Nicholas's book brings establishment to another faction, the free-living nematodes. As such it further frustrates the attempts of those who would study nematodes as a single biological grouping; it does, however, recognise the important role of the new nematologists who specialise in 'free-living' forms. 'Free-living' is a difficult notion; essentially it describes what is left when the parasitic species are taken away. Such a definition has hazy edges: parasites often have 'free-living' larvae, fungal feeders are free-living, but species which feed on higher plants are parasitic. Dr Nicholas wisely does not attempt strict definitions and emphasises soil, fresh water and marine forms.

As a series of essays some chapters can be recognised as those in which Dr Nicholas has had direct personal experience—such as 'Culture and Nutrition', Cytogenetics and Development' and 'Biochemistry'—and these achieve a high level of comprehension and accuracy. The chapter on 'Ecology' is a clear and thoughtful assemblage of a very heterogenous subject area, and is the best short review of the subject currently available. One of the most attractive features of the book is that marine nematodes are not neglected. Furthermore, they are set apart from other free-living species on basic biological criteria but at the right distance.

In the first chapter on Morphology and Structure, much of the information is drawn from nematodes other than free-living forms. This has introduced some patchiness and has resulted in such important sections as those on the sense organs, excretory system and cryptobiosis being weaker than the other more restricted parts of the book. References are quoted but they are not necessarily the most relevant; they do not always demonstrate a synthesis made by the author before their selection. The book is eminently readable and the few typographical errors and mis-spelled names are more distracting than misleading. The book is informative rather than scholarly. Dr Nicholas has chosen a chatty style which narrates the subject rather than one that provokes the reader to think beyond these pages. Typical of this are his useful chapters on taxonomy and methods of handling and culturing.

There are over 1000 references but only 20 are quoted after 1971 and none after 1973. This has resulted in certain topics, in which rapid growth has occurred, already being dated; it is, however, more contemporary than any other competing text.

Nematodes are being widely used as model metazoans by geneticists, behaviourists, gerontologists, and developmental biologists. This is the best book available to give these researchers and their students in allied disciplines a 'worm's-eye view' of the nematodes. ☐



Male fiddler crab (*Uca maracoani maracoani*), Trinidad

Fiddler crabs

R. G. Hartnoll

Fiddler Crabs of the World: Ocypodidae—Genus Uca. By Jocelyn Crane. Pp. xxiii+736. (50 plates.) (Princeton University: Princeton, New Jersey, November 1975.) £43.70.

WITH over seven hundred large pages, and weighing in at only a shade under 3 kg, this is a truly impressive book at first sight. It is a pleasure to report that this favourable opinion is reinforced by a study of the contents. This monograph covers all the known species of *Uca* in respect of taxonomy, functional morphology and behaviour. To a great extent it is based on many years of the author's own work in various regions of the world, and as a consequence the different topics are integrated to an unusual degree.

The first and larger part of the book is the systematic section within which each of the 62 species of *Uca* is dealt with in turn. For each one there is a full morphological description together with an analysis of its differences from related species, followed by details of behaviour, distribution and type material. I was relieved to find that the genus *Uca* was not fragmented here, for although large it has a distinctiveness and homogeneity which render it an excellent example of the generic concept. Recently Bott (*Senckenberg. biol.*, **54**, 315–325; 1975) has proposed the division of *Uca* into nine genera, but it now seems unlikely that this partition will be generally accepted. Dr Crane renders the genus manageable by introducing a new scheme of subgenera, and simplifies a number of taxonomic difficulties by a judicious use of subspecific categories. Her analysis of criteria for erecting subspecies is particularly interesting.

The second part of the book contains a series of chapters which collate and discuss, subject by subject, the information presented species by species in the first. There are excellent chapters on zoogeography, environmental relationships and functional morphology, but to me the most interesting are those on behaviour and evolution within the genus. Few large genera have been analysed in such detail with regard to these latter.

There are a number of appendices, of which the most useful are the keys to species in different geographical areas, and that providing practical advice on the observation, collection, transport and maintenance of these crabs. There are many high quality line drawings and photographs which amplify both the morphological and behavioural accounts. Overall this is a remarkably comprehensive review of a most interesting genus, and has been produced to a very high standard. It will be of great interest to carcinologists of a variety of disciplines, and it is only a pity that the economics of publishing must put it beyond the reach of most. ☐

Biology of man . . .

Cedric A. B. Smith

Human Variation and Natural Selection. (Symposia of the Society for the Study of Human Biology. Volume 13). Edited by D. F. Roberts. Pp. ix + 209. (Taylor and Francis: London, 1975). £4.50.

THIS book consists of a selection of papers read at meetings of the Society for the Study of Human Biology about 15 years ago, and published in previous symposia about that time. In spite of the lapse of time since then these papers still have freshness and relevance. A considerable amount of detailed knowledge has accumulated in the topics dealt with in many of the papers since they were written; but the basic ideas have not changed very much, even though the emphasis may now be different.

The first topic of importance essentially concerns the factors shaping the relative frequencies of various inherited characters in a natural population. There are contributions on that by L. S. Penrose, A. R. G. Owen, C. A. Clarke, P. M. Sheppard and T. Dobzhansky. They explain three main ways of maintaining variability in a population, namely by heterozygote advantage, by mutation counter-balanced by natural selection, and by natural selection favouring different types in different habitats. There are three main ways of introducing genetic variability for a limited time: genetic drift, and the response to changing natural selection, either because of a temporary change (for example, an epidemic) or a permanent shift in environment. Fifteen years ago we knew one clear case of heterozygote advantage, the sickle-cell polymorphism in Africa, described by Allison in a later paper in this book, and a number of cases of balance between mutation and selection. Although theory has been developed much further, observational knowledge remains substantially as before.

Another group of papers, by A. E. Mourant, A. C. Allison, N. A. Barnicot and S. M. Gartler, examines various Mendelian characters which show polymorphism, such as blood groups, haemoglobins, haptoglobins, transferrins and amino acids. Except for certain polymorphisms, such as that of abnormal haemoglobins, which seem to be related to the presence of malaria, these characters seem suitable for anthropological investigations designed to find which populations are most closely related. Later work has extended this list, especially as regards enzyme variability.

Drawing of a spectral tarsier. Taken from *Uniqueness and Diversity in Human Evolution: Morphometric Studies of Australopithecines.* By Charles E. Oxnard. Pp. viii + 133. (University of Chicago: Chicago and London, 1975.) £9.

There are also papers by A. C. Stevenson on "Biological studies of small communities". E. H. Ashton on "Rate of change in Primate Evolution", S. B. Holt on "Dermatoglyphic Patterns", and G. A. Harrison on "Pigmentation".

Each paper has had added to it a few paragraphs indicating more recent developments in the field. □

. . . and baboons

T. H. Clutton-Brock

Social Dynamics of Gelada Baboons. (Contributions to Primatology, Volume 6.) By Robin and Patsy Dunbar. Pp. viii + 157. (Karger: Basel, London and New York, 1975.) SFr.89; DM 85; $37.25.

To casual inspection, the social system of geladas closely resembles that of hamadryas baboons: the population is divided into stable harem units usually consisting of a single mature male, around three adult females and their offspring. Younger males join unisexual troops and both harems and all-male groups form bands which share a common home range. The takeaway message of the Dunbars' monograph is that superficially similar social systems may be the product of different behavioural arrangements. In the hamadryas, harem units are maintained by the chauvinistic activities of the male, who herds females and enforces group cohesion. In the geladas a more egalitarian situation prevails and harem units are maintained by strong social bonds between females.

The monograph provides a detailed but readable description of the anatomy of gelada social organisation. Its claim to cover group dynamics is less well justified. The study was of relatively short duration and few individuals could be recognised. As a result, virtually all interpretation of group dynamics is based on selected evidence. A more fundamental shortcoming (although one which has applied to many primate field studies) is the lack of theoretical orientation. This leads to a number of unfortunate effects: extensive description of trivial or obvious aspects of social behaviour, persistent attention to null hypotheses which are palpably false, and inadequate discussion of points of particular interest. The book will be of major interest only to primatologists. A stronger theoretical bent could have led to a study which was of much wider significance. □

Male gelada baboon

ARDEA Photographics

Molecular biology of RNA phages

J. Hindley

RNA Phages. Edited by Norton D. Zinder. Pp. viii+428. (Cold Spring Harbor Laboratory: Cold Spring Harbor, 1975.) n.p.

RNA Phages is the latest of the Cold Spring Harbor Monograph series and provides 400 pages of endeavour devoted largely to our understanding of the structure, replication, and translation of the phage genome. The RNA phages are among the smallest known viruses and since the techniques for RNA sequence analysis preceded those for DNA, they formed the logical choice for the new art of the sequencer. This emphasis is apparent in many of the contributions and adds a comfortable sense of reality to the descriptions of the various interactions which regulate the replication and translation of the viral RNA.

The fourteen chapters of this book provide a reasonably well balanced selection of current work and interest in this field. Invariably there are some omissions, and some are serious, for example the work of Weissmann *et al.* on the *in vitro* synchronised synthesis of both plus and minus RNA strands; and the extension of such methods in site-directed mutagenesis gets negligible attention. Similarly the important research of Porter *et al.* on the rebinding of $Q\beta$ initiator fragments to *Escherichia coli* ribosomes fails to get any mention whatever in spite of their particular relevance to the problem of ribosome recognition of initiator regions in phage RNA.

The first chapter provides an authoritative account of the physical properties of RNA phages and their RNAs, and the second contribution reviews the genetic studies which established the presence of three cistrons (complementation groups) in RNA phages.

One of the most informative features contributing to the study of RNA phages is the ability to compare primary RNA sequences with the amino acid sequences of the phage-specified proteins. The third chapter reviews our knowledge in this direction and clearly brings out the value of such work in pin-pointing the initiation triplets, the apparent non-random use of synonymous codons, the location and effects of amber mutations and the explanation of the 'read through' phenomenon found in phage $Q\beta$.

The fourth chapter surveys the physiology of phage attachments and penetration, and makes the point that much remains to be done in understanding these phenomena. Nearly 100 pages in the succeeding four articles are devoted to the replication of phage RNA. This makes fascinating reading and the main impression one is left with is the exquisite adaptation of the replicase complex, which contains both virus- and host-specified proteins, to the selective and controlled replication of the viral RNA. No less interesting is the elaborate control exercised on the translation of the viral RNA and this topic is discussed in the next three chapters. Again it is the level of organisation and sophistication achieved in these, the simplest of phages, which excites the wonder of the reader. The *tour de force* of the book comes in chapter thirteen in which Fiers presents the entire RNA sequence of the A-protein gene of phage MS2 and shows the value of such detail in grappling with the problems of gene organisation.

To complete the picture the morphogenesis of RNA phages, and the fact that *E. coli* is not unique in acting as host to RNA phages, are discussed in two informative contributions.

The clear message of this book is that the molecular biology of RNA phages have much wider implications into the general problems of replication and translational control, and it can be recommended to the student and specialist alike. □

Paracelsian chemistry

M. P. Crosland

The Chemists and The Word: The Didactic Origins of Chemistry. By Owen Hannaway. Pp. xiii+165. (Johns Hopkins University: Baltimore, Maryland and London, November 1975.) £6.50.

When did chemistry emerge as a distinct discipline? Hermes, Tresmegistos, Moses, Aristotle, Jabir, Paracelsus, Boyle, Lavoisier and Liebig have all been candidates with their respective supporters for the title of founder of chemistry. Even if we refuse to take the earlier names in this list seriously we cannot ignore a group of contemporary historians of science who have made great claims for Paracelsus and (more convincingly) for some of his followers. They have argued that the chemical philosophy of the Paracelsians in the sixteenth and seventeenth centuries was not only fundamental to the development of chemistry but to science generally. It sought to comprehend all natural phenomena and thus provided an alternative to both the traditional philosophy of Aristotle and the new mechanical philosophy of Descartes. Yet the obscurantism and secrecy of some Paracelsians have hardly helped to support this claim. Their apparently arbitrary use of language made any dialogue with outsiders extremely difficult. Dr Hannaway appreciates the relevance of the Paracelsians but admits that there was an essential difference between their aims and methods and that of a rational science. He therefore attempts a synthesis and tries to pin-point the beginning of the science of chemistry in a reaction to Paracelsianism. His hero is Andreas Libavius who first appears as the enemy of Paracelsian chemistry, seen as a "counter-culture". But it was the same Libavius who became the propagandist for a new chemistry, presented in a systematic and logical form.

A full discussion of the various strands of Paracelsianism would require a book of massive proportions and we can accept the author's decision to focus on one writer, Oswald Croll, as a representative of the Paracelsian school. This choice enables him to expose some of the deficiencies of the Paracelsians and to contrast their approach with that of Libavius. Hannaway suggests that chemistry emerged not so much from Paracelsian alchemy as from a more positivistic attitude of those who tried to present a new and rational science in textbook form. This is a plausible argument but the author goes further in claiming that the writing of one particular textbook, the *Alchemia* (1597) of Libavius constituted the "invention" of chemistry. It is easier to interpret the role of textbooks in science as providing a consolidation of a new approach rather than the main instrument of a "scientific revolution", and a detailed discussion of Ramism is included to help support this argument.

I would like to be able to recommend the book to any working chemist who felt that the didactic origins of his subject were worthy of some consideration. It would be fairer, however, to recommend the book rather as a contribution to the intellectual history of the sixteenth century. With its detailed textual analysis it is more a book for the specialist. Readers may not be convinced that the work of Libavius alone marked the beginning of 'real' chemistry but the discussion provides a persuasive reminder of the metaphysical and religious context of the emergence of early modern science. □

Reference work on rabies

G. S. Turner

The Natural History of Rabies. Edited by George M. Baer. Volume 1: Pp. xvi+454. $43.00; £21.50. Volume 2: Pp. xvi+387. $36.00; £18.00. (Academic: New York and London, September–October 1975.)

THESE two volumes provide a comprehensive central source of information on many facets of this ancient disease. They examine the molecular biology of the virus, the pathogenesis and pathology of rabies, its diagnosis, epidemiology and its prevention in man. The contributors of the 42 chapters have in many cases either been members of, or advisors to, the World Health Organisation (WHO) expert committee on rabies. Readers active in rabies research will recognise many familiar footprints in well trodden ground.

Whether the contents always justify the title of 'natural history' is arguable since this implies a continuing record of natural phenomena. Advances in rabies research seem to be discontinuous—much of the mythology was dispelled by Pasteur but more than 60 years elapsed before the successful application of cell culture methods enabled the virus to be seen for the first time, and also permitted the first reasonably accurate estimate of its size, the physicochemical and biological properties of which are described in part I. Similar methods, described in chapter 9, enabled mechanisms of infection and growth at the cellular level to be examined.

The section on the pathogenesis of rabies recalls arguments concerning the respective roles of neural and haematogenous spread of virus to the central nervous system (CNS). Evidence is also discussed for extraneural replication of virus before and after invasion of the CNS Latent and abortive rabies is reviewed here as well as the effects of interferon on infection. Contributors remind us, however, that the scanty histological changes observed in infected animal brain, contrast strikingly with the severe clinical signs observed in rabies, and that its pathogenesis remains "a complicated and little understood series of events".

The section on laboratory diagnosis details techniques which are already described elsewhere (see WHO monograph series No. 23, *Laboratory Techniques in Rabies*). Surprisingly the passive haemagglutination test for rabies antibody does not appear here.

Almost half the second volume is devoted to epidemiology. Individual chapters provide abundant information on rabies in each of many wild and domestic animals in both natural and experimental conditions. Wildlife data concerns several species indigenous only to the new world. No doubt the omission of global epidemiology reflects difficulties in acquiring information from underprivileged countries in which rabies is endemic.

The chapters devoted to the control of rabies in dogs, cats and other domestic animals seem to prefer vaccination to other means, Although vaccination is undeniably effective it is not a control method likely to be favoured in England at the moment. Included in the methods reviewed for the control of wildlife rabies are population reduction, vaccination, the use of gametocides and more recently the use of anticoagulants for destroying vampire bats. The methods mostly suggest directions for future research rather than immediate solution of this intransigent problem.

The final chapters deal with rabies in man, its prevention, first aid treatment, diagnosis and clinical manifestations. Recent optimistic hopes of cure of the overt disease by aggressive intensive care are also mentioned. Here too, details are given of the passive immunisation of man with anti-rabies serum as well as a highly critical review of human, postexposure vaccine therapy and the difficulties of assessing its efficacy. An appendix gives a guide to specific treatment.

Not every contribution is a model of stylistic or verbal excellence and the occasional mistake has escaped the proof reader. This is probably inevitable in a work of this magnitude and in no way detracts from its value as the best and most practical reference text on the subject that has appeared in recent times. □

Endocrine system in perspective

K. Fotherby

Comparative Vertebrate Endocrinology. By P. J. Bentley. Pp. xi+415. (Cambridge University: Cambridge, London and New York, January 1976.) Cloth £12; Paperback £4.95.

ENDOCRINOLOGY is a fascinating subject and to those of us who deal mainly with human endocrinology this fascination is increased by taking a broader view and looking at the way in which the endocrine system has evolved. For this purpose this textbook is ideal. It presents the subject matter in a readable way emphasising the differences between species from a functional aspect. It not only contains a wealth of information concerning the endocrinology of the various species but also contains more than enough basic information concerning the biosynthesis, metabolism and mode of action of the hormones for the book to be read and understood without the necessity of referring to a textbook of basic endocrinology.

In addition to chapters on the comparative morphology of endocrine tissues and the chemical structure and polymorphism and evolution of hormones, there are chapters dealing with the relationship between endocrinology and nutrition, calcium metabolism, the integument, osmoregulation, and reproduction. The text is illustrated with many excellent diagrams.

Although there are species differences in the morphology of the endocrine tissues, and in the chemical structure and biological and immunological behaviour of the hormones, many of these variations are such that they can often be placed into categories corresponding to the major systematic groups of vertebrates. These species differences suggest an orderly evolutionary change. The pathways of hormone synthesis, mechanisms of secretion and mode of action seem to be similar in all vertebrates although the nature of the stimulus responsible for the secretion of a particular hormone may vary according to the physiological role in which the hormone may be involved.

The longest chapter in the book is that devoted to reproduction. This is not surprising since the reproductive process shows a wide diversity of morphological and physiological characteristics, and the latter requires the co-ordination of many different events which are controlled by a number of different hormones. In spite of the wide variation in the morphological and physiological aspects of reproduction, the nature of the hormones involved varies little. The gonadal steroid hormones have the same structure in all vertebrates. More variation is shown by the gonadotrophins; and it is of interest that in most tetrapods the pituitary secretes two gonadotrophins, luteinising hormone and follicle-stimulating hormone, whereas in some vertebrates e.g. fishes, only a single gonadotrophin is secreted.

Although written as a textbook for graduate and undergraduate courses in comparative endocrinology, it should be read by all interested in endocrinology. It will be useful as a reference book for research workers, and the availability of a paperback edition may ensure a wide sale for this excellent book. □

All you need to know about H–2

Hilliard Festenstein

Biology of the Mouse Histocompatibility-2 Complex: Principles of Immunogenetics Applied to a single system. By Jan Klein. Pp. xii+620. (Springer: Berlin and New York, 1975.) DM97.60; $42.

During the past decade, the science of immunogenetics as applied to the major histocompatibility system, previously regarded as a somewhat esoteric area of scientific endeavour, has come into its own. Its rate of development has been unprecedented and it has provided new insight into such diverse biological problems as transplantation and cancer immunology at one end of the spectrum to those which are not even immunological at all—like hormonal control and the genetics of infertility—at the other end of the spectrum It represents the key to the understanding of antigen recognition, cell interaction, embryonic development and differentiation, and processes involved in the pathogenesis and manifestation of a variety of diseases. It has become important not only for the basic scientist but also for clinicians—rheumatologists, neurologists, nephrologists, obstetricians, endocrinologists, to mention but a few.

The major histocompatibility system is the most complex and polymorphic system geneticists have had to cope with and the more that is known about it, the more important it seems to become. Jan Klein's exhaustive treatise on the immunogenetics of H–2 is thus a very welcome and timely arrival It starts with an introduction dealing with the history of transplantation immunogenetics and the geneology and origin of the inbred and congenic laboratory mouse strains; there follow detailed descriptions of the methods and the current status of H–2 serology In the section dealing with histocompatibility, there is a short introduction to transplantation immunology followed by a discussion on the methods of histocompatibility testing and effects, comparing those of the H–2 system with various others—the erythrocyte (Ea) alloantigen loci, Ly, virus-associated alloantigen loci and non-H–2 histocompatibility loci

The section on the principles of genetic analysis, recombination and mutation in the H–2 complex and the genetics of the H–2 linked loci will be of particular value to those researchers entering the field; it contains a clear account of the genetic terminology and approach basic to an understanding of this science There is also a comprehensive section on the biochemistry of the H–2 gene products. The book is reasonably up to date and even includes discussions on the recently discovered complement gene in H–2 and the Ia specificities, as well as the newly described *X* locus. It ends with a short speculative chapter on the function and evolution of the H–2 complex; and there is a chronological table of the major events in the history of the H–2 system which is interesting and puts the author's own contributions into perspective. The references are an invaluable part of this book and will provide researchers with a rapid and comprehensive list of the major publications in this fascinating and new field.

This book will probably find a place on the shelf of every laboratory seriously engaged in the study of immunogenetics. □

Indispensible guide to tropical pollution

C. M. Yonge

Tropical Marine Pollution. (Elsevier Oceanography Series, 12.) Edited by E. J. Ferguson-Wood and R. E. Johannes. Pp. ix+192. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.65; $26.95.

We may be grateful for the original idea of the late E. J. Ferguson-Wood resulting in the production of this book under the final editorship of R. E. Johannes, who is also responsible for writing much the most important sections.

Although temperate seas have been the initial sufferers from pollution, the basically more susceptible tropical waters are all too rapidly becoming similarly afflicted. And what may be effective preventive measures in the former regions may not be applicable in the tropics. In so many different ways involving chemical and physical characters, community structure and biological processes, responses to pollution in the tropics differ significantly from those in temperate waters.

The second and longest chapter comprises the fullest available study of pollution and degradation of coral reef communities Writing with a great wealth of experience, Dr Johannes covers every aspect of the problem from the effects of sedimentation to those of dredging, from those of bad land management to those of sugar mill discharge and of sewage outflow, the latter so dramatically demonstrated at Kaneohe Bay to the north of Honolulu where I once collected from immaculate reefs. He finally discusses effects of oil and thermal pollution, and those of abnormal salinities. These last are produced by desalination plants, an analysis by R. H. Chesher of the impact of one at Key West forming the final chapter.

What is known about the impact of man on mangroves—it is estimated that some of those defoliated during the Vietnam war may take a century to recover—is succinctly reviewed by W. E. Odum and R. E. Johannes. There is a most useful account by J. C. Zieman of the effect of pollution on tropical eel grass communities, aptly described as "nurseries, food producers and stabilisers of the sea floor." The same author also collaborated with Ferguson-Wood in a description of the effects of thermal pollution by a power station most unsuitably sited in Biscayne Bay, Florida and involving the destruction of both sea grass beds and mangroves.

Although there is much of value in all chapters, those contributed by Dr Johannes are of over-riding importance greatly enhanced by his personally assembled list of references occupying 28 pages. This is a short and expensive book but is contains a wealth of information valuable to all, but surely indispensable to those confronted by the almost daily growing problems of pollution in tropical seas. □

Multidisciplinary approach to pollution

N. W. Moore

Pollutants and Animals: A Factual Perspective. By F. Moriarty. Pp. 140. (Allen and Unwin: London, September 1975.) £5.65.

This short book is a lucid account of the facts which have to be taken into account by those who study the effects of pollutants on living organisms. Probably more is known about the pollution effects of the organochlorine insecticides than any other chemical group, and so Dr Moriarty uses the organochlorines as the example on which to base his pertinent conclusions. About 80% of the book is about these compounds and the chemically related polychlorobiphenyls (PCBs), the remaining 20% being devoted to the effects of heavy metals, and a brief general review of pollution in air, water and on land. Radioisotopes are covered in two pages. At first sight the subject matter of the book seems unbalanced, but the advantages of using the organochlorines as an example outweigh this disadvantage.

Pollutants and Animals is accurate, clearly written and very readable Technical terms such as axon, isomer and isotope are defined and so it can be read by the informed layman as well as by scientists with a professional interest in pollution research and control, or in teaching or learning about pollution. Dr Moriarty shows how ideas have progressed in recent years, and, by pointing out significant gaps in knowledge, also succeeds in putting over the authentic 'feel' of doing research in this field. Studies which should be extended include those on the possible biological effects of pesticide metabolites, the behaviour of DDT in mud, the extent to which DDT is converted into DDE and transformed to PCBs in the physical environment, and laboratory studies on the interactions of different pollutants on fish.

Dr Moriarty's comments on the limitations of the LD$_{50}$ test are salutary for those who rely too much on this particular indicator of hazard. The desirability of obtaining more residue data when toxicological tests are made, is another practical requirement to which Dr Moriarty draws attention.

Conventional wisdom has it that organochlorine insecticides always become concentrated in food chains. In his critique of the pioneer work of Hunt and Bischoff at Clear Lake, Dr Moriarty shows how that hypothesis has had to be modified in the light of subsequent research. Similarly, it is commonly believed that populations of birds of prey have declined as the result of sublethal effects of organochlorines. Dr Moriarty, in a perceptive review of this very complicated subject, shows how lethal and sublethal effects have varied in their relative importance between different species.

This book should be of great interest to those concerned with the organisation of science. It demonstrates not only the desirability, but the necessity of relating chemistry, toxicology and population dynamics in order to provide the scientific base for pollution control. The multidisciplinary team of which Dr Moriarty was a distinguished member, was a casualty of the split of the Nature Conservancy. Some way must be found of supporting similar teams in the future. Anyone reading this excellent book will come to appreciate why. □

Food from the sea

G. E. Fogg

Marine Photosynthesis: With Special Emphasis on the Ecological Aspects. (Elsevier Oceanography Series, 13.) By E. Steemann Nielsen.) Pp. ix+141. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.52.00; $21.75.

PROFESSOR Steemann Nielsen has contributed more than any other person to our knowledge of photosynthesis in the sea. His introduction in 1952 of the radiocarbon technique gave us a simple and sensitive means, which has been very widely used, for measuring the rate of photosynthesis in natural waters; and his laboratory experiments have deepened our understanding of the manner in which the photosynthetic activity of phytoplankton organisms varies in response to conditions in the sea. In this book, the first to be devoted to a kind of photosynthesis which provides at least half the Earth's supply of food materials, he brings together these contributions and sets them against the background of modern ideas about the mechanism of the photosynthetic process and its evolutionary history.

This background is sketched in the first two chapters; there follow discussions of underwater daylight, the units in which irradiance should be expressed, the kinds of photosynthetic organisms in the sea, the effects of various factors on their photosynthetic activity, the interrelationships of photosynthesis and respiration, and physiological adaptation to irradiance and temperature. The later chapters deal with photosynthesis *in situ,* and the primary production of the sea and its importance to man. It is a pity that in this account the author should have confined himself rather strictly to presenting his own work and avoided discussion of controversial matters such as the importance of extracellular products of photosynthesis.

Several independent groups of investigators now believe that they have demonstrated that healthy populations of natural phytoplankton frequently release as much as half of their photosynthetic product directly into the surrounding water. This much is briefly mentioned but after a longer discussion of the errors which may arise in the determination of extracellular products of photosynthesis the reader may be left with an impression, which the facts do not actually justify, that this important phenomenon is no more than an experimental artefact. Similarly, there is insufficient consideration of the implications of experiments by other workers on temperature adaptation.

Nevertheless, this is a wide-ranging and authoritative account which will be valuable and stimulating to students and research workers alike. □

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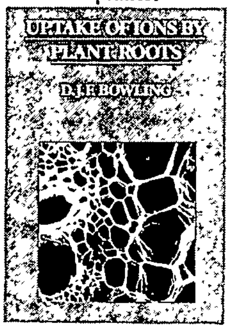

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Biological problems of the deep sea

N. B. Marshall

Physiological Aspects of Deep-Sea Biology By A. G. Macdonald. Pp. xi+450. (Cambridge University: Cambridge and London, October 1975.) £18.50.

MONOGRAPHS of the Physiological Society " . . . review and assess important and neglected areas of physiology". The physiological aspects of this monograph are centred largely on the effects of hydrostatic pressure, which is certainly an important and neglected area of physiology. After the early experiments of Regnard and Certes in the 1880s, little was done until the late 1920s and 30s, as may be seen by reference to Cattells' (1936) *Biological Review*. The more recent and considerable advances in our knowledge of the effects of pressure on organisms are evident in Symposium XXVI (1972) of the Society of Experimental Biology and in an American Symposium (1972) entitled *Barobiology and the Experimental Biology of the Deep Sea* (Univ. North Carolina). Dr Macdonald was a contributor to both symposia. Besides his concern with the effects of pressure on the activity and metabolism of oceanic animals, he has also been involved in the design of apparatus for recovering deep-sea animals without pressure change.

In his brief introduction to the physical nature and inhabitants of the deep sea, the author devotes most space to the few species of animals, such as the ostracod *Gigantocypris* and the pogonophoran worm *Siboglinum*, that have been used by physiologists. There follow chapters on the molecular, biochemical, and physiological aspects of high hydrostatic pressure. Between these chapters and one entitled Deep-Sea Bio-engineering there are two dealing with sensory physiology and buoyancy in the deep sea and deep-sea nutrition

In his competent reviews of the effects of pressure from the molecular to organ system level, it is clear from more than the bibliography that most of the author's research has been on whole animals. When dealing with molecular aspects, he concludes that high pressure is of considerable importance in the molecular biology of deep-sea organisms but adds these cautionary words: "The volume changes and pressure effects which have been described were largely derived from *in vitro* systems and we have eventually to tackle the functional, living state. It is conceivable that a great deal of pressure sensitivity may disappear when an isolated protein is returned to its physiological environment. It is equally possible that the pressure sensitivity of intact animals will be greater than their isolated parts through amplification processes at the kinetic and integrated levels of organisation." Later he remarks that ". . . the intact organism may possess compensatory mechanisms which are absent in an isolated organ. Experiments with intact animals will help to identify some of their pressure-sensitive components." These quotations also show that the author writes well (although he could be more of a 'which hunter').

Thirty years ago, Sir Frederick Russell foresaw that physiologists would be eager to study deep-sea organisms when proper facilities were available. That physiologists are now able to take their equipment to sea is abundantly plain in Dr Macdonald's review of sensory physiology and buoyancy. Perhaps the outstanding contribution of other sea-going physiologists towards a proper understanding of deep-sea nutrition has been to measure rates of metabolism. How, for instance, do the changing pressures and temperatures that are experienced during vertical migrations affect the rate of oxygen consumption of deep-sea animals? Recently, *in situ* respirometry of benthic communities and benthopelagic fishes has shown that the pace of deep-sea life is much slower than that in shallow waters. But deep-sea conditions can be simulated in the laboratory, and what can be achieved by high pressure systems is well reviewed by Dr Macdonald in his last chapter, which also deals with the physiology of deep-sea man and the roles of deep-sea vehicles and surface ships.

In a comprehensive book of this kind errors are bound to occur, but one that is presumably not due to the author concerns the caption of Fig. 1 21 on page 28. The photograph is not of a rat-tail (macrourid) but of a chimaerid species.

The book contains a full bibliography (up to 1975). The references for each chapter are kept separate but 'pressurised' together at the end of the book, which makes transfers from textual references to bibliographies irritating to the reader Finally, I agree with the publishers' statement that "The book will be of great value to all interested in marine physiology and the biological problems of the deep sea" but wonder how many of "all interested" will be able to buy it. ☐

articles

α-Chain contacts in the polymerisation of sickle haemoglobin

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Five new double-mutant haemoglobins composed of β^S chains and α chains with different substitutions, which are located at the surface of the tetramer, have been prepared. Although all the hybrids are more soluble than deoxyhaemoglobin S, the individual differences between these molecules make it possible to evaluate several regions on the α chains for intermolecular contacts in the polymerisation of deoxyhaemoglobin S.

THE discovery that the aggregation of deoxyhaemoglobin S (HbS) involves the formation of tightly packed helical rods[1,2] shows that multiple contact points must cooperate in the formation of the individual helices as well as in the interaction between adjacent tubules to give tactoids[3]. Identification of these contact points is facilitated by investigating the behaviour of haemoglobins which, in addition to the β^6 valine mutation, have others elsewhere on the surface of the molecule. Only two of these have so far been investigated—HbC Harlem[4,5] and HbS Memphis—and in both cases the additional substitution diminishes the tendency to sickle[4-6]. This implicated β^{73} and α^{23} in contact formation between adjacent molecules. To widen this approach we have prepared five new double-mutant haemoglobins which carry the β^6 valine substitution together with an additional one on the α chains. This facilitates a direct comparison of the effect of changing specific residues on the α chain on the properties of haemoglobins carrying the β^S substitution.

Preparation of hybrid haemoglobins

Haemoglobin was prepared from fresh human blood as described previously[7]. The mutant haemoglobins used in this investigation are listed in Table 1. HbS was obtained from a single homozygous donor by exchange transfusion. It was isolated by chromatography on CM–Sephadex (C-50) using a salt gradient of 0→0.1 M NaCl in 0.05 M phosphate buffer, *p*H 6.7. All the other mutant haemoglobins were separated from the haemolysates on DEAE–Sephadex (A-50) with a *p*H gradient of Tris buffer[13]. After column chromatography the haemoglobin solutions were concentrated using Amicon or collodion membranes. The purity of the isolated mutant haemoglobins was checked by acrylamide gel electrophoresis[14].

Hybrid molecules containing two β^S chains and two α chains derived from the mutant haemoglobin were prepared by incubating the α-chain mutant haemoglobin with a twofold excess of HbS in 0.2 M acetate buffer, *p*H 4.8, for 4 h at 0 °C as described previously[15]. The hybrids were again isolated by chromatography on DEAE or CM–Sephadex. HbS alone was subjected to identical procedures to serve as a control.

The identity and purity of the hybrids was established by electrophoresis both of the intact hybrid haemoglobins[16] and of the individual polypeptide chains[17]. Desarg HbS was prepared as described for Desarg HbA[18]. One mol of (arginine+ornithine) was liberated per αβ dimer and the protein was homogeneous on acrylamide gel electrophoresis. The oxygen equilibrium curves of all haemoglobins, including the hybrids, were measured at 20 °C and *p*H 7.30 as described previously[19,20]. Solubility was determined as a function of ionic strength in phosphate buffer, *p*H 6.8, by a modification of Itano's method[21,22] both in the presence and absence of dithionite. This method was chosen, rather than gelation measurements because it requires much

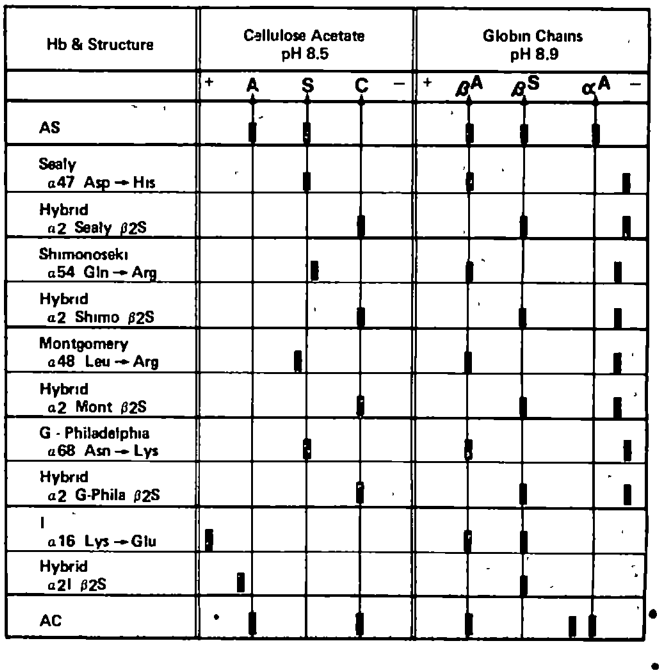

Fig. 1 Electrophoretic patterns of native and hybrid haemoglobins and their constituent globin chains on cellulose acetate. Tris-EDTA borate (TEB) buffer, *p*H 8.5, for haemoglobins; TEB buffer, *p*H 8.9, containing 2-mercaptoethanol and 6 M urea, for globins.

Table 1 Properties of mutant haemoglobins

Mutant haemoglobin	Structure	Ref.	% Total haemoglobin
Hb Sealy (hasharon)	$(\alpha^{47\ \mathrm{Asp} \rightarrow \mathrm{His}})_2\beta_2{}^A$	8	20
Hb Shimonoseki	$(\alpha^{54\ \mathrm{Glu} \rightarrow \mathrm{Arg}})_2\beta_2{}^A$	9	24
Hb Montgomery	$(\alpha^{48\ \mathrm{Leu} \rightarrow \mathrm{Arg}})_2\beta_2{}^A$	10	23
HbI	$(\alpha^{16\ \mathrm{Lys} \rightarrow \mathrm{Glu}})_2\beta_2{}^A$	11, 12	14
HbG Phila	$(\alpha^{68\ \mathrm{Asn} \rightarrow \mathrm{Lys}})_2\beta_2{}^A$	9	100*
HbSG Phila	$(\alpha^{68\ \mathrm{Asn} \rightarrow \mathrm{Lys}})_2(\beta^{6\ \mathrm{Glu} \rightarrow \mathrm{Val}})_2$	9	21

*The blood of this patient contained only HbG Phila and Hb H.
The latter was precipated quantitatively leaving HbG Phila in solution.

smaller amounts of material. Studies of chemically modified HbS have shown that these two methods give parallel results[23].

Characterisation of double-mutant molecules

Hybridisation between HbS and the α-mutant haemoglobins can be represented by the following equation:

$$\underset{\alpha\ \text{mutant}}{\alpha_2{}^X\beta_2{}^A} + \underset{\text{HbS}}{\alpha_2{}^A\beta_2{}^S} \rightleftarrows \underset{\text{Hybrid}}{\alpha_2{}^X\beta_2{}^S} + \underset{\text{HbA}}{\alpha_2{}^A\beta_2{}^A}$$

The electrophoretic mobilities of all the native and hybrid haemoglobins are shown in diagrammatic form in Fig 1. Four of the mutant haemoglobins, that is, haemoglobins Sealy, Shimonoseki, Montgomery and G Phila, have the same charge as HbS. Therefore, only three components are isolated after hybridisation, that is, the hybrid and HbA in equal amounts and a fraction of intermediate mobility containing both HbS and unchanged α mutant. In the case of HbI, on the other hand, all four components of the hybridisation mixture can be eluted separately. The hybrid haemoglobins comprised from 15 to 20% of the total. The electrophoretic patterns of the constituent globin chains of both the native haemoglobins and the hybrids are also shown in Fig. 1. The α chains of the four haemoglobins that resemble HbS electrophoretically all move cathodically to α^A, but the α chain of HbI moves anodically to α^A. The mobility of the α^I globin happens to be the same as that of β^S, so that α^I and β^S form only one band. None of the hybrid haemoglobins showed any appreciable contamination with α^A or β^A globin chains.

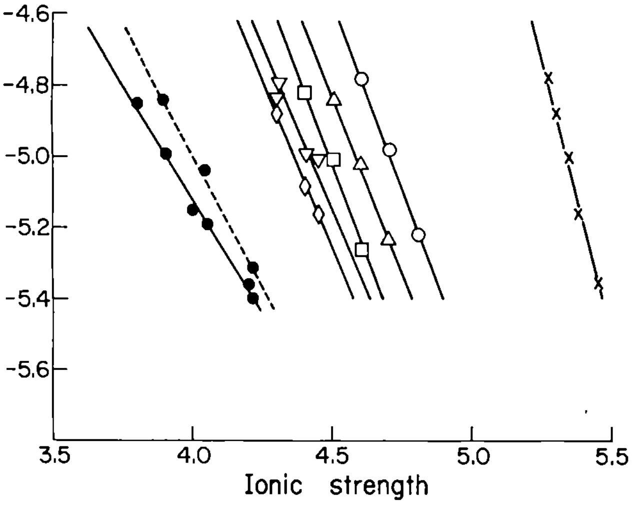

Fig. 2 Solubility of HbS and double-mutant haemoglobins. All measurements in potassium phosphate buffer, *p*H 6.8, at 25 °C. The solubility is given in mol of Hb tetramer per l of solution in the presence of solid phase. ●–●, S deoxy; ● – – ●, desarg S deoxy; ×, S oxy; ○, $\alpha_2{}^{\mathrm{Sealy}}\ \beta_2{}^S$ deoxy; △, $\alpha_2{}^{\mathrm{Shimo}}\ \beta_2{}^S$ deoxy; □, $\alpha_2{}^{\mathrm{Mont}}\ \beta_2{}^S$ deoxy; ▽, $\alpha_2{}^{\mathrm{G\,Phila}}\ \beta_2{}^S$ deoxy; ◇, $\alpha_2{}^I\ \beta_2{}^S$.

The structural and functional integrity of the hybrid tetramers was tested in several ways. (1) They bind oxygen cooperatively (n=2.4–2.6) and with a normal oxygen affinity. (2) HbS reisolated after hybridisation with HbA has the same solubility as the untreated protein. (3) In the case of one of the hybrids, that is $\alpha_2{}^{\mathrm{GPhila}}\beta_2{}^S$, it was possible to compare the tetramer prepared *in vitro* by hybridisation of HbG Phila and HbS with a naturally occurring one isolated from the blood of a patient who is heterozygous for HbG Phila and homozygous for HbS[8]. Since the natural and the synthetic hybrid gave the same result (Table 2) it can be assumed that the procedure for preparing the other hybrids has no effect on their solubility *per se*.

Solubility of mutant sickle haemoglobins

The effect of different substitutions in the α chain on the solubility of deoxyhaemoglobin S is shown in Fig. 2. Clearly all the double-mutant haemoglobins are significantly more soluble in strong salt than deoxyhaemoglobin S. This is in sharp contrast to the parent α-mutant haemoglobins which all have the same solubility as deoxyhaemoglobin A, that is their solubility is 10^{-5} M at an ionic strength of 5.70.

Since the most characteristic property of HbS is the large difference in the solubility of the oxy and deoxy forms, this value is used in Table 2 to compare the solubilising effect of the various α-chain mutations. Their influence on the solubility of the hybrid molecules must be due to interference with the polymerisation characteristic of deoxyhaemoglobin S because no solubilising effect of the α-chain substitution is found in the parent haemoglobin.

It is striking that a chemical modification of the α chains which favours the oxy conformation of haemoglobin[18], that is, removal of the C-terminal arginine residues, has little effect on the solubility of deoxyhaemoglobin S (Fig. 2).

The inhibitory effects of the different α-chain substitutions on the aggregation of HbS are not simply related to the alteration in charge. Thus, the Sealy and G Phila hybrids have the same charge and a very different solubility while the HbI and G Phila hybrids are almost equally soluble, but differ by six charges per tetramer.

It therefore seems that the synthetic hybrids represent a new approach to locating contact points provided by the α chains in the polymerisation of deoxy HbS. Such information is essential to restrict the number of possible orientations of the tetramers within the helix[24–27].

Fig. 3 Schematic model of the haemoglobin molecule showing the locations of the amino acid substitutions.

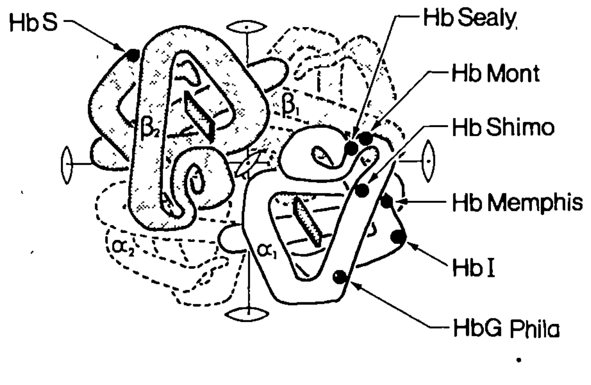

Table 2 Effect of deoxygenation on solubility

Haemoglobin	Position of α substitution	Ionic strength at which the solubility is 10^{-5} M Oxy	 Deoxy	 (Oxy–deoxy)
$\alpha_2{}^{A}\beta_2{}^{S}$		5.33	3.90	1.43
$\alpha_2{}^{\mathrm{Sealy}}\beta_2{}^{S}$	47	5.28	4.70	0.58
$\alpha_2{}^{\mathrm{Shimonoseki}}\beta_2{}^{S}$	54	5.28	4.58	0.78
$\alpha_2{}^{\mathrm{Montgomery}}\beta_2{}^{S}$	48	5.43	4.48	0.95
$\alpha_2{}^{\mathrm{G\ Phila}}\beta_2{}^{S}$ (synthetic)	68	5.50	4.42	1.08
$\alpha_2{}^{\mathrm{G\ Phila}}\beta_2{}^{S}$ (natural)	68	5.50	4.42	1.08
$\alpha_2{}^{I}\beta_2{}^{S}$	16	5.50	4.35	1.15

The positions of the α substitutions are shown in Fig. 3. It should be noted that all of them are on or near the surface of the molecule. It is clear, however, that there is considerable variation between the effect of the different substitutions on polymerisation. The largest increase in solubility is observed with $\alpha_2{}^{\mathrm{Sealy}}\beta_2{}^{S}$ with a substitution in the CD bend. Therefore, an intermolecular bond is probably located at this position. Moreover, the magnitude of the effect makes it likely that this bond is responsible for a large portion of the contact energy in this region. Conversely, the small increase in solubility due to the replacement of α^A by α^I chains suggests that here the substitution breaks only one of many bonds which participate in this contact region. This is in agreement with the conclusions reached by Levinthal *et al.*[28]. Figure 2 shows that the hybrids with α chains from haemoglobins Sealy and Montgomery have significantly different solubilities, although the substitutions are on adjacent loci (α47 and α48). Inspection of the three-dimensional model of haemoglobin reveals, however, that although residue 47 is on the surface of the molecule pointing outwards, residue 48 is oriented in the opposite direction.

The α mutants available to us have only permitted the preparation of hybrids with substitutions as far as the E helix (Fig. 3). We hope that gifts of α mutants with substitutions elsewhere on the α chain will make it possible to explore contact points in the remainder of this chain.

This work was supported by grants from the US Public Health Service, the US National Science Foundation and the National Foundation–March of Dimes. R. B. is a recipient of a research career award from the National Heart and Lung Institute. For blood samples we thank Drs Jack B. Alperin, Thomas Hosty, Mary Jane Grey, James Wolff, and Ronald Rieder.

Received December 8, 1975; accepted January 30, 1976.

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Bacterial cloning of plasmids carrying copies of rabbit globin messenger RNA

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DNA copies of rabbit globin mRNA have been inserted into the plasmid mini-ColE1. After addition of poly(dT) tails, the plasmid was used to prime the copying of mRNA by reverse transcriptase. Mini-ColE1 carrying globin sequences were denatured and annealed with mini-ColE1 tailed with poly(dA). Functional plasmids containing the synthesised globin sequences were isolated by transfection in E. coli.

IT has been demonstrated that eukaryotic DNA can be cloned in bacteria after insertion into plasmids[1–4] or bacteriophage genomes[5]. These experiments indicate that it should be possible to clone any eukaryotic gene in bacteria and identify those bacteria carrying the gene by screening procedures involving hybridisation with radioactive messenger RNA (mRNA). This cloned DNA will facilitate the study of the sequence organisation and structure of eukaryotic genes. At present, bacterial cloning of defined eukaryotic protein-specifying genes has only been achieved with the multiple-copy histone genes[6]. The greatest proportion of genes, however, are found as single copies in the eukaryotic genome (for review see ref. 7).

Unless substantial purification of such genes can be achieved *in vitro*, cloning of individual plasmid-gene chimaeras (made from unfractionated eukaryotic DNA) will be difficult since specific clones are likely to be present in the population at the

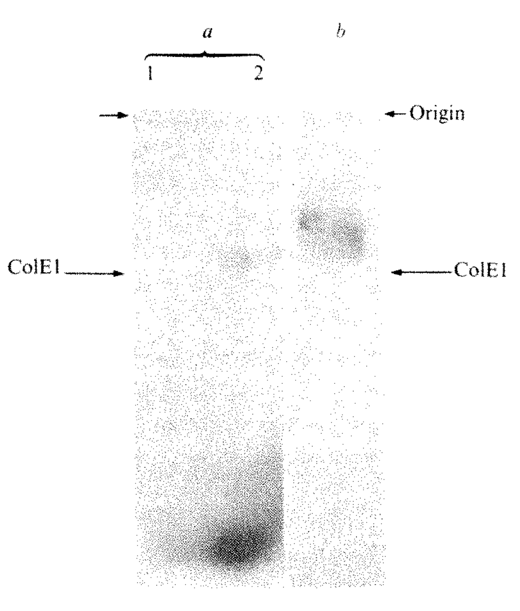

Fig. 1 Analytical Agarose gel electrophoresis of terminal transferase and reverse transcriptase activity with mColE1 DNA. *a*, 0.5 μg intact mColE1 DNA (panel 1) or 0.5 μg *Eco*RI-digested mColE1 DNA (panel 2) were incubated with 1 μCi ^{32}P-dTTP (New England Nuclear, 100 Ci mmol^{-1}) and 2.5 μg terminal transferase (P.L. Biochemicals, Lot No. 34421, specific activity 5,260 U mg^{-1}) in 10 μl reaction volume containing 100 mM potassium cacodylate (*p*H 7), 7.5 mM potassium phosphate (*p*H 7), 8 mM $MgCl_2$, 2 mM 2-mercaptoethanol. After 1 h at 37 °C, the reaction was terminated by addition of EDTA to give 10 mM and sucrose was added to 20% (w/v): the mixture was loaded on a 1% Agarose (w/v) vertical slab gel. Electrophoresis was for 1 h at a constant current of 40 mA. (The gel buffer was 20 mM sodium acetate, 40 mM Tris-acetate (*p*H 8.3), 0.2 mM EDTA.) An autoradiograph of the gel was made using Kodak Auto-Process X-ray film. Unreacted ^{32}P-dTTP appears (near the anode) at the bottom of the autoradiograph. *b*, 2 μg mColE1 DNA tailed with poly(dT) (prepared as in legend to Fig. 2) plus 1 μg rabbit globin mRNA were incubated in 50 μl containing 50 mM Tris-HCl (*p*H 8.3), 20 mM dithiothreitol, 6 mM $MgCl_2$, 60 mM NaCl, 500 μM dATP, dCTP, dGTP, dTTP, 1 μCi ^{32}P-dCTP, 2.5 μg actinomycin D and 0.16 μg reverse transcriptase (specific activity 29,316 U mg^{-1}). After 2 h at 37 °C the reaction mixture was diluted with an equal volume of 0.6 M NaCl, 0.02 M sodium acetate (*p*H 5) and passed over a small column (in a 1-ml plastic pipette) of Sephadex SP-50 to remove the unreacted nucleotides. The breakthrough peak was precipitated with ethanol (−20 °C, 18 h). The precipitate was dissolved in gel buffer, electrophoresed and the gel autoradiographed as in Fig. 1*a*. The anode is at the bottom of the gel.

level of 1 cell in 10^6. Methods for isolating clones such as the modified sib selection procedure used to identify bacteria carrying plasmids with histone genes[6] is unattractive for low frequency genes since the number of screening selections would need to be very large. A report by Rougeon *et al.*[8] has demonstrated the insertion of double-stranded DNA copies of rabbit globin mRNA into the plasmid pCR1. Using different methods from those described by Rougeon *et al.*[8], we have inserted double-stranded complementary DNA copies of rabbit globin mRNA into the plasmid mini-ColE1 (mColE1)[9]. The method involves a minimum of manipulations and should be suitable for producing such plasmids from any mRNA containing poly(A). The procedure is a modification of the A–T tail method[10,11] and utilises poly(dT) tails, added to mColE1, as primer for reverse transcriptase copying of globin mRNA into DNA. Double-stranded globin DNA is produced by repair *in vivo* of the single-stranded complementary DNA (cDNA) in the plasmid.

Preparation of plasmids containing globin DNA

The plasmid mColE1 possesses a single site for cleavage by the restriction endonuclease *Eco*RI (ref. 9). Digestion of the plasmid with this enzyme therefore yields a uniform population of linear molecules with an identical point of breakage. It has previously been shown that the enzyme terminal transferase adds nucleotides to 3′-termini of double-stranded DNA molecules[10–12] but that the efficiency of the process is greatest in the presence of a free 3′-OH group. Nevertheless, the ability of terminal transferase to add dT-residues to the unmodified ends of *Eco*RI-digested mColE1 was tested. A sample of mColE1 digested with *Eco*RI was incubated with terminal transferase plus ^{32}P-dTTP. The products were analysed by Agarose gel electrophoresis (Fig. 1*a*). The enzyme adds dT residues to the DNA to produce a heterogeneous size range of elongated molecules (Fig. 1*a*, panel 2). Intact circular plasmid DNA shows no change when incubated with terminal transferase (Fig. 1*a*, panel 1). The size range of the poly(dT) tails was rather difficult to control, however, and this heterogeneity is presumably due to the poor and unsynchronised initiation of elongation.

The ability of the mColE1 molecules carrying poly(dT) tails to act as primer for reverse transcriptase copying of globin mRNA was tested. This transcription would be analogous to the conventional preparation procedure of cDNA from mRNA (for example see ref. 13). Fig. 1*b* shows that a further elongation of the mColE1 resulted from the reverse transcriptase activity. Thus DNA copies of globin mRNA had been added (covalently attached) to the mColE1 by reverse transcriptase.

These observations showed that a population of plasmid molecules terminated by segments of globin DNA could easily be prepared and led to the development of a procedure for the cloning of plasmids carrying these globin sequences. The general protocol for this preparation is given in Fig. 2. The procedure consisted of five steps. (1) Circular mColE1 was digested to completion with *Eco*RI; (2) the digested plasmid was tailed with either poly(dT) or poly(dA); (3) the plasmid tailed with poly(dT) was used to prime the synthesis of cDNA using globin mRNA as a template; (4) the plasmid molecules containing cDNA were further tailed with poly(dT); (5) the resultant population from step 4 was mixed with plasmid molecules tailed with poly(dA) and the mixture denatured and annealed. Transfection was carried out with *Escherichia coli*. Colicin-resistant clones were plated on nutrient agar and grown overnight at 37 °C. *E. coli* clones containing globin plasmids were finally screened by hybridisation.

The annealing procedure was carried out in two parts. First, plasmid DNA from step 4 (that is, carrying globin DNA and a further poly(dT) tail) was mixed with an excess of plasmid DNA tailed with poly(dA). This mixture was denatured and annealed at high temperature and high DNA concentration to allow specific hybridisation of the plasmid DNA strands. Second, the DNA mixture was diluted to about 5 μg ml^{-1} before incubation at 37 °C to facilitate hybridisation of the poly(dA) tail of one strand with the poly(dT) tail present at the end of the globin DNA segment on the opposite strand. Such dilution of the DNA is known to facilitate intramolecular hybridisation of poly(dA) and poly(dT) (that is circularisation) rather than intermolecular hybridisation[14]. Although plasmid circularisation was not demonstrated directly, it was found that production of transfectant *E. coli* was absolutely dependent on the final 37 °C annealing step. Since the reverse transcription was carried out in the presence of actinomycin D, only single-stranded globin DNA would be synthesised. It is possible that the transfection efficiency could have been improved by repair *in vitro* of the single-stranded region and subsequent *in vitro* ligation. Since globin-plasmids could be produced without such *in vitro* steps, however, repair and ligation of the plasmids was allowed to occur *in vivo*.

Identification and characterisation of bacteria carrying globin plasmids

The first stage in the identification of individual clones containing globin plasmids was growth of the transfected cells in the presence of colicin. Only cells carrying mColE1 plasmids

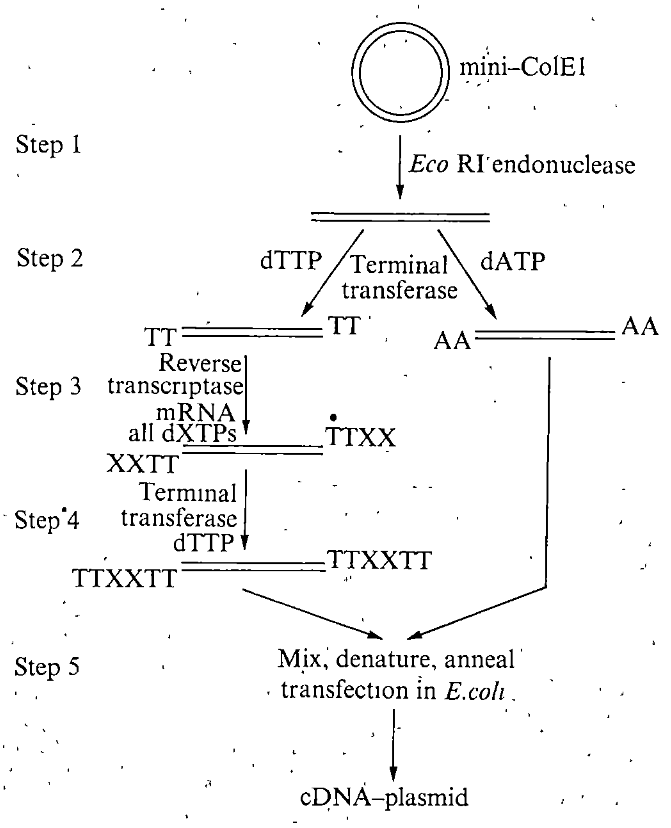

Fig. 2 General scheme for production of globin plasmids. Step one: mColE1 was prepared by the procedure described[15] and *Eco*RI was prepared by the method of Yoshimori[16]. mColE1 DNA (20 μg) was digested with *Eco*RI (the quantity of enzyme necessary was previously determined by digestion of λ phage DNA) in 100 μl of 10 mM Tris-HCl (*p*H 7.5), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol for 2 h at 37 °C. The reaction was terminated by freezing. Step two: two aliquots of 50 μl from the *Eco*RI digestion mix (10 μg DNA) were added to 50 μl containing 100 mM potassium cacodylate (*p*H 7), 7.5 mM potassium phosphate (*p*H 7), 8 mM $MgCl_2$, 2 mM 2-mercaptoethanol. dTTP was added to one aliquot and dATP to the other to give 0.3 mM. The tube containing dTTP also contained 10 μCi ^{3}H-dTTP (New England Nuclear) so that the labelled plasmid DNA could easily be monitored in subsequent steps. 10 μg terminal transferase were added to each mixture and the tubes were incubated at 37 °C for 1 h. Again the mixes were frozen to terminate the reaction. At this stage, an analytical Agarose gel was routinely carried out to check the efficiency of the transferase reaction. Step three: the mColE1 tailed with poly(dT), prepared in step 2 (10 μg DNA), was incubated in a 300-μl volume containing 20 μg rabbit globin mRNA (prepared by oligo(dT) chromatography of rabbit reticulocyte polysomal RNA[17]), 5 μg actinomycin D, 10 μCi ^{3}H-dCTP (New England Nuclear, 28 Ci $mmol^{-1}$), 500 μM dATP, dCTP, dGTP and dTTP in the same buffer as described in the legend to Fig. 1*b*. Reverse transcriptase (1 μg) was added and the mixture incubated at 37 °C for 2 h. The mix was further incubated with 10 μg pancreatic ribonuclease (previously boiled to remove contaminating deoxyribonuclease activity) for 30 min at 37 °C, extracted once with an equal volume of phenol and passed over a small column of Sephadex SP-50 (in a 1-ml plastic pipette) in 0.3 M NaCl, 10 mM sodium acetate (*p*H 5). One-drop fractions were collected and the position of the DNA monitored by scintillation counting of 1-μl aliquots. The peak fractions were pooled and precipitated overnight at −20 °C by the addition of two volumes of ethanol. (Note: XTP refers to equal quantities of each nucleotide triphosphate.) Step four: the ethanol-precipitated mColE1 containing globin DNA sequences (prepared in step 3) was spun down, dried *in vacuo* and dissolved in 50 μl of 100 mM potassium cacodylate (*p*H 7), 7.5 mM potassium phosphate (*p*H 7), 8 mM $MgCl_2$, 2 mM 2-mercaptoethanol and 0.3 mM dTTP. Terminal transferase (6 μg) was added and the mix incubated at 37 °C for 1 h. Step five: after 1 h incubation in step 4, 50 μl (10 μg) of mColE1 tailed with poly(dA) (prepared in step 2) was added to the reaction mixture and the volume was increased to 250 μl with the salt concentration adjusted to 100 mM NaCl, 10 mM Tris-HCl (*p*H 8), 10 mM EDTA. The solution was boiled for 5 min and incubated at 70 °C for 30 min, after which 2.75 ml of the above buffer were added and incubation continued for 5.5 h at 37 °C. Finally the solution was extracted once with an equal volume of phenol and precipitated overnight at −20 °C by the addition of 2 volumes of ethanol in the presence of 50 μg *E. coli* tRNA carrier. The final dried pellet was dissolved in 300 μl of 30 mM $CaCl_2$, 10 mM NaCl, 0.5 mM EDTA, 10 mM Tris-HCl (*p*H 8) (transfection buffer).

Competent cells were prepared (as described by Cohen *et al.*[18]) from an overnight culture of *E. coli* C600 $r^- m^-$ (a gift from Dr Noreen Murray). Approximately 1.2 × 10^8 competent cells were pelleted and suspended in 300 μl of solution containing the annealed mColE1 population. (Control transfections consisted of 1 μg native mColE1 in 300 μl transfection buffer or 300 μl transfection buffer with no DNA.) The bacterial suspension was held on ice for 60 min, heated at 42 °C for 2 min and held on ice again for 60 min (with an occasional shake). 0.7 ml 2 × TY medium (1.6% (w/v) tryptone, 1% (w/v) yeast extract 0.5% (w/v) NaCl *p*H 7.4) was added and the bacteria incubated at 37 °C for 45 min. Sufficient colicin was added to kill 1.2 × 10^8 cells (the dosage was previously determined) and the suspension incubated for a further 30 min at 37 °C. The cells were then pelleted, resuspended in 0.3 ml 2 × TY medium containing half the above quantity of colicin and plated on nutrient agar plates overnight at 37 °C.

should be resistant. Colicin treatment of bacteria, however, does not result in the full extinction of all non-plasmid containing bacteria and further, the annealing procedure will lead to the presence of plasmids tailed only with poly(dA) and poly(dT). The identification of clones carrying globin plasmids was facilitated by hybridisation as follows. Sixty-four randomly chosen colicin-resistant clones were grown in 1-ml cultures. Eight separate pools of eight clones were made and these pools were grown overnight in 100-ml cultures. DNA was prepared from these cultures and tested for the presence of globin-plasmids by hybridisation with globin ^{3}H-cDNA (prepared by normal reverse transcription). Table 1*a* shows the results of this initial screening: of the eight pools tested, half showed the ability to hybridise ^{3}H-cDNA and thus the presence of globin plasmids. The percentage of cDNA hybridised in each case was around 30–40% of the total cDNA recovered. Since the ^{3}H-cDNA used to assay for positive clones was a mixture of α-and β-globin sequences, 40% hybridisation approaches the proportion of the ^{3}H-cDNA representing the α- or β-globin sequence. Identification of individual positive clones was carried out simply by growing the eight individual clones of any positive pool in 100-ml cultures, preparing DNA from these cultures and identifying the positive clone(s) by cDNA hybridisation. For example, Table 1*b* shows the identification of one clone containing a globin plasmid from the eight pooled clones of the initial culture number *C*. It seemed that in this pooled culture *C*, only one clone actually contained a globin plasmid; thus, about 7% of the clones resistant to colicin actually contain globin plasmids. Most of the remainder of colicin-resistant clones (amounting to about 10 times as many clones as in a mock-transfection) are presumably due to plasmids formed from the reannealing of strands tailed with poly(dA) and poly(dT) but without added globin sequences.

The globin plasmid identified above was used in further characterisation studies. To determine whether the globin plasmid contained sequences for α or β globin, plasmid DNA was hybridised with either ^{3}H-cDNA prepared from the original mRNA (a mixture of α- and β-globin sequences) or with ^{3}H-cDNA prepared from mRNA originating from the rabbit reticulocyte post-ribosomal supernatant (this mRNA is enriched in α-globin mRNA[20]). The total cDNA population was found to hybridise with the globin plasmid to around 47% whereas the α globin enriched cDNA hybridised only about 22% (Table 2). Since β-globin sequences probably predominate in the total mRNA population but are relatively depleted in the α-enriched mRNA, the DNA in the plasmid seems to be the complement of β-globin mRNA. Furthermore, when annealed with unmodified mColE1, ^{3}H-cDNA of total globin and α-enriched globin only bound about 9% and 4% respectively to the hydroxyapatite (Table 2). This percentage is the amount of ^{3}H-cDNA consistently found to bind to hydroxyapatite in the absence of hybridisation. Thus there are no sequences in

Table 1 Identification of clones containing globin plasmids

	Culture no.	*c.p.m. hybridised	†Recovered counts in hybrid (%)
Initial pools of eight clones *a*	*A*	5	5
	B	0	2
	C	618	43
	D	400	30
	E	500	35
	F	529	37
	G	0	1
	H	36	4
Individual clones of culture *C* *b*	1	0	0.4
	2	0	0
	3	2,923	31
	4	50	5
	5	6	0
	6	0	0
	7	0	0
	8	0	0

*c.p.m. hybridised = c.p.m. in 0.4 M sodium phosphate fraction (after hydroxyapatite fractionation) minus c.p.m. in 0.4-M fraction of the control tube containing 100 μg mColE1 DNA.

† % of recovered c.p.m. in 0.4-M fraction minus % c.p.m in 0.4-M fraction of control tube

a, After overnight growth of transfectant *E. coli* on nutrient agar plates in the presence of colicin (see legend to Fig. 2), 64 colonies were randomly selected for growth in 1 ml of 2 × TY broth. Eight pools of eight 1-ml cultures were made in 100 ml 2 × TY, grown to $A550 = 0.9$ and chloramphenicol added to a concentration of 200 μg ml^{-1}. Growth was continued overnight at 37 °C. The cells were collected and cleared lysates prepared as described[15]. DNA was precipitated for 1.5 h at 4 °C after addition of NaCl to give 750 mM and polyethylene glycol 6,000 to give 15% (w/v). The precipitates were drained, dissolved in 1 ml 50 mM NaCl and digested at 37 °C for 30 min with 10 μg pancreatic ribonuclease; 5 μg proteinase K (Merck) was added to each tube and digestion continued for 30 min at 37 °C. At the end of this time, 10 μl 10% SDS were added, plus 1 ml 2 M NaCl, 10 mM Tris-HCl (*p*H 8). The DNA was extracted 3 times with equal volumes of phenol and chloroform (1:1 ratio). The final aqueous phase was sonicated for 2 × 30 s and the DNA precipitated by the addition of two volumes of ethanol (−20 °C for 2 h). The precipitates were dried and dissolved in 0.2 ml water, placed in a small siliconised tube with 2,000 c.p.m. ^{3}H-globin cDNA (prepared by conventional oligo(dT) priming of mRNA as in ref. 19: s. a. 6 × 10^6 cm^{-1} μg^{-1}) and sodium phosphate buffer (*p*H 6.8) to give 0.24 M (total volume 0.3 ml). These mixtures were boiled for 5 min and incubated for 15 h at 70 °C. The hybridisation was assayed by hydroxyapatite fractionation as described[19]. A control hybridisation tube containing 100 μg sonicated mColE1 DNA and ^{3}H-cDNA was processed to determine background binding of ^{3}H-cDNA in 0.4 M phosphate on hydroxyapatite fractionation. *b*, The individual clones of culture *C* were grown in 100 ml culture as described for *a*. DNA preparation and hybridisation (against 10,000 c.p.m. ^{3}H-cDNA) were also as in (*a*).

mColE1 DNA which hybridise with ^{3}H-globin cDNA.

The globin plasmid described will contain tracts of poly(dA) and poly(dT). Therefore, it was necessary to eliminate the possibility that the hybridisation experiments were detecting hybrid formation between the poly(dA) of the plasmid and the poly(dT) tract known to be present at the end of ^{3}H-cDNA[19]. Two control hybridisation experiments were carried out. The globin-plasmid DNA was annealed with mouse immunoglobulin light-chain ^{3}H-cDNA. This cDNA will contain only poly(dT) tracts in common with sequences present in the globin plasmid. Table 2, line 6 shows that 9.6% of this cDNA binds to the hydroxyapatite after annealing with the globin plasmid compared to 5.6% binding after annealing with unmodified mColE1 DNA. Thus the poly(dT) tracts of the ^{3}H-cDNA do not seem to be involved in hybridisation described. Furthermore, the ability of ^{3}H-globin cDNA to hybridise with the globin plasmid in the presence of tenfold excess of unlabelled poly(dT) competitor was examined. Table 2, line 2, shows that about 45% hybridisation occurs in the presence of poly(dT), therefore the hybridisation of the ^{3}H-cDNA is essentially unaffected by the presence of the poly(dT). This observation confirms that the hybridisation observed was due to DNA sequences complementary to globin mRNA present in the plasmid. The length of the globin segments in the plasmids is as yet unknown. It is likely, however, that the population as a whole shows a similar heterogeneity to the cDNA population made by conventional reverse transcriptase copying of mRNA. Thus an individual plasmid may contain a sequence anywhere from 50 residues to the entire length of the globin mRNA.

This paper describes the insertion of DNA copies of rabbit globin mRNA into the plasmid mColE1 and subsequent amplification by cloning in *E. coli*. These DNA copies are probably partial copies of the globin mRNA and represent the successful synthesis of part of the globin gene. A similar synthesis has also been described elsewhere[8]. The method should be applicable to any poly(A)-containing RNA or RNA to which poly(A) can be added *in vitro*, so that a large supply of the sequence can be made available. It is hoped that the ability to clone globin sequences in bacteria will facilitate isolation, by hybridisation procedures, of those regions from the chromosomal DNA involved in control of globin gene activity. For example, the plasmid DNA could be covalently linked to cellulose to provide a basis for hybridisation/chromatographic purification of the complementary regions from nuclear DNA. The principal advantages of this method are the large quantity of DNA available, plus the important point that the plasmid contains double-stranded DNA which will allow purification of both strands of the chromosomal DNA in a single step.

In addition, manufacture of double-stranded copies of messenger RNA in mColE1 will make sequencing of such

Table 2 Hybridisation properties of purified globin plasmid

	DNA hybridised	^{3}H-cDNA type	c p.m. hybridised (%)
(1)	Globin plasmid	Total globin	49.2
(2)	Globin plasmid	Total globin (in presence of poly(dT))	45.1
(3)	mColE1	Total globin	9.8
(4)	Globin plasmid	α-Enriched globin	22.7
(5)	mColE1	α-Enriched globin	3.9
(6)	Globin plasmid	Mouse Ig	9.6
(7)	mColE1	Mouse Ig	5.6

Globin plasmid DNA was prepared as described for the mColE1 DNA (Table 1) All DNA stocks were sonicated for 1 min before use. ^{3}H-cDNA was prepared from rabbit reticulocyte 9S mRNA (total globin cDNA) from rabbit reticulocyte post-ribosomal 9S mRNA (α-enriched cDNA) and from mouse myeloma MOPC21 light chain mRNA (mouse Ig cDNA) as described[19]. In each hybridisation tube, 2 μg of DNA were annealed with 10,000 c.p.m. ^{3}H-cDNA for 16 h at 70 °C in 300 μl 0.24 M sodium phosphate buffer (*p*H 6.8) after boiling for 5 min (Tube 2 included 10 μg poly(dT) (P.L. Biochemicals)). After annealing, the mixes were diluted with 3 ml 0.12 M phosphate containing 100 μg *Micrococcus* DNA and fractionated on 0.5 gm hydroxyapatite (BioRad HTP) as described[19] % c.p.m. hybridised represents the percentage of the total recovered counts in the 0.4-M fraction from the hydroxyapatite column.

species easier by application of the rapid sequencing methods now emerging[21]. In fact, it is conceivable to clone any available RNA species (for example, eukaryotic ribosomal RNAs) by first adding poly(A) to the RNA[22,23] and then inserting DNA copies in mColE1: the inserted DNA copies would be more amenable to sequence studies than the native RNA itself.

I thank Dr J. W. Beard for supplying reverse transcriptase, Dr C. Milstein for discussion and Mr A. Forster for technical assistance.

Addendum

Although the experiments described in this paper were, in the main, carried out before publication of the US National Institutes of Health Committee report on containing experiments with recombinant DNA, the guidelines suggest that the containment facilities used for such experiments should be mentioned in publications. It thus seems relevant to report that containment equivalent to P2 level was used initially for these experiments. Classification of this particular experiment under the NIH guidelines is rather difficult but nevertheless since publication of the guidelines, work has been carried out in conditions satisfying P3 containment.

Received January 9; accepted February 24, 1976.

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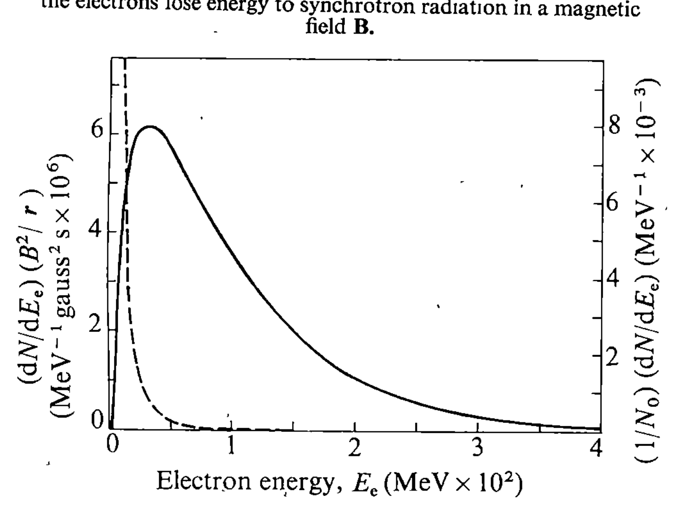
letters to nature

Annihilation of matter and antimatter and the cosmic X-ray background

IN cosmological models where matter and antimatter occur symmetrically in the Universe[1–5], their annihilation is likely to take place also at the present time. At the annihilation of a proton and an antiproton, ~ 1.6 negative electrons and an equal number of positrons, each with an energy of ~ 10–100 MeV are produced (Fig. 1), together with γ rays and neutrinos[6]. The initial electron energies can be expected to decrease gradually because of their interaction with cosmic magnetic fields, plasmas, and electromagnetic radiation. As a result of these interactions synchrotron radiation, bremsstrahlung, and inverse Compton radiation are all emitted. When optical photons are scattered against the relativistic electrons, X rays are produced by the inverse Compton effect. This mechanism may be an important source of cosmic X rays. Our purpose here is to study how efficient this X-ray mechanism can be in intergalactic space (where most of the annihilation electrons are expected to be found) and to see whether it can offer an explanation to the cosmic X-ray background observed (see refs 7–9).

Fig. 1 ——, Energy spectrum of electrons (negative and positive) directly-produced by proton–antiproton annihilation. The spectrum is normalised by means of the total number of electrons N_0 formed. — — —, Stationary energy spectrum of annihilation electrons $\mathrm{d}N/\mathrm{d}E_e$ multiplied by B^2/r. The spectrum is obtained for a constant rate of annihilation r when the electrons lose energy to synchrotron radiation in a magnetic field **B**.



For the sake of clarity we shall consider a simplified model of the X-ray mechanism assuming:

(1) That the annihilation of protons and antiprotons occurs at a constant rate giving rise to relativistic electrons with a production spectrum according to Fig. 1, with a maximum close to 30 MeV.
(2) That the energy of the electrons decreases continuously, primarily from synchrotron losses, so that a stationary energy spectrum is obtained for the electrons.
(3) That the primary optical radiation in intergalactic space (with which the annihilation electrons interact and produce X rays) consists mainly of galactic light having an intensity of $S_{10} = 1.4$ ($m_v = 10$) star degree^{-2} (ref. 10) and a spectrum with the shape of a black-body distribution at a temperature $T_{ph} = 6 \times 10^3$ K.

For assumptions (1) and (2), Ekspong *et al.*[6] have estimated the stationary energy spectrum of the annihilation electrons (Fig. 1). A considerable concentration of electrons towards small energies can be noticed in the graph. It should be mentioned that the shape of this spectrum is unchanged if one includes energy losses from inverse Compton scattering of the electrons against black-body radiation of low energy.

Korshak[11] has calculated the spectrum of the inverse Compton radiation produced by one electron of energy E_e interacting with black-body photons of temperature T_{ph} and density n_{ph}. He finds

$$P_C(\nu) = 1.24\, \sigma_T c h n_{ph} F(\nu/\nu_C) \text{ erg s}^{-1} \text{ Hz}^{-1} \qquad (1)$$

provided $E_e \gg m_e c^2$, $\beta E_e \gg h\nu \gg kT_{ph}/\beta$, $(\beta = v/c \simeq 1)$.

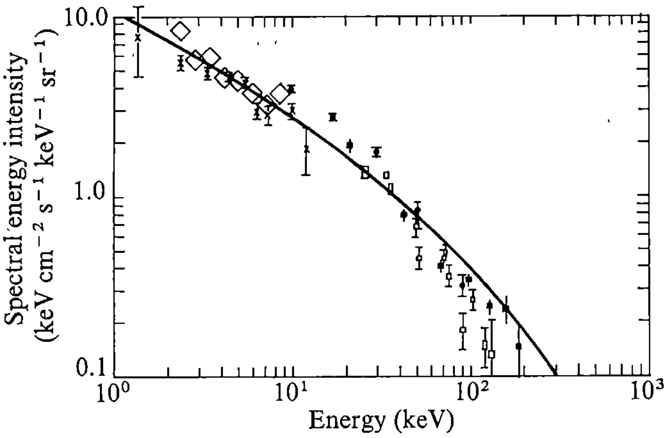


Fig. 2 Spectrum of inverse Compton radiation resulting from scattering of galactic light on annihilation electrons which decrease in energy because of synchrotron radiation (solid curve). The inverse Compton spectrum is compared with measured intensities of the cosmic X-ray background in the energy interval 1 keV $\lesssim h\nu \lesssim 10^2$ keV (ref. 9). The experimental points are from: ●, OSO III; ◇, LLL; ×, ASE; □, Leiden-Nagoya; ■, GSFC-OSO V.

$\sigma_T = 6.65 \times 10^{-25}$ cm^2 is the Thomson cross section, ν is the frequency of the scattered photons, and

$$\nu_C = (4kT_{Ph}/h)\,(E_e/m_e c^2)^2.$$

The function $F(\nu/\nu_C)$ reaches its maximum value of 0.254 at $\nu \simeq \nu_C$ and is $\simeq 1.65\nu/\nu_C$ for $\nu/\nu_C \ll 1$ and

$$\simeq (\nu/\nu_C)\exp(-\nu/\nu_C) \text{ for } \nu/\nu_C \gg 1$$

By integrating numerically the spectrum defined by equation (1) over the stationary energy distribution of the relativistic electrons (dashed curve in Fig. 1) we can estimate the spectrum of the inverse Compton radiation in our model, which is shown in Fig. 2, together with the measured intensities of the cosmic X-ray background in the interval 1 keV $\lesssim h\nu \lesssim 10^2$ keV (ref. 9). As is clear from Fig. 2, the inverse Compton spectrum agrees well with the observed spectrum in this interval.

It should be noted that except for the total intensity there is no adjustable parameter in this derivation. For photon energies > 1 MeV, the inverse Compton spectrum starts to deviate appreciably from observational data[7]. The maximum photon energy of the inverse Compton radiation that can be produced by our mechanism is well above 10 MeV.

The intensity of the inverse Compton radiation is proportional to the density of the primary photons and to the density of the relativistic electrons. Furthermore, the intensity depends also on the size of the volume which the electrons occupy. With the radius of the volume being $R = 10^{10}$ light yr $= 10^{28}$ cm and the primary radiation consisting of galactic light as assumed in (2), the intensity in Fig. 2 is obtained for a mean electron density $n_e \simeq 10^{-9}$ cm^{-3} in the energy interval 10 MeV $< E_e < 10^2$ MeV. This electron density is about two orders of magnitude smaller than the mean atom density of galactic material throughout the Universe.

The electrons which are necessary to produce the cosmic X-ray background will of course also give rise to other types of electromagnetic radiation. One of these is the synchrotron radiation whose intensity and frequency is influenced by the magnetic field **B**. To obtain a stationary spectrum for the electron energies of interest, $E_e \gtrsim 1$ MeV (as assumed in (2)), the magnetic field must be so strong that the decay time (τ_s) arising from synchrotron losses

$$\tau_s = \frac{5.1 \times 10^8}{B^2}\,\frac{1}{1+E_e/m_e c^2}\ \text{s} \qquad (2)$$

(B in gauss) is at least less than the age of the Universe, being $\sim 10^{10}$ yr $= 3 \times 10^{17}$ s. Thus, the magnetic field must be $\gtrsim 2 \times 10^{-5}$ gauss.

With a magnetic field of this strength, the intensity of synchrotron radiation in the radio band produced by the annihilation electrons necessary to generate the X rays, would be higher than experiment allows[12–14]. The solution of this problem may be that the annihilation electrons are created locally in quasars and galactic nuclei[15], where the magnetic field is sufficiently strong to cause a rapid decay in the energy of the electrons. This could also lead to absorption of the γ rays formed by annihilation, so that their flux does not become unacceptably high[16,17]. The annihilation electrons, on the other hand, escape into intergalactic space where they become uniformly distributed and where they produce the X-ray background by interacting with optical photons.

We have for simplicity disregarded effects of cosmological evolution, but, nevertheless, our description may be adequate in a framework such as the Klein–Alfvén metagalactic theory. A more detailed discussion has to wait for general development of the symmetric cosmology.

We thank Professor H. Alfvén for suggesting this problem as well as for a number of discussions.

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Received October 14, 1975; accepted February 9, 1976.

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X-ray observations of GX17+2 and GX9+9 by Aryabhata

X-RAY observations on GX17+2 and GX9+9 were made using the proportional counter telescope on board the first Indian satellite 'Aryabhata'. We present these results, which enable us to start discussing source mechanisms for these objects.

The proportional counter had an effective area of 15.4 cm^2 and was fitted with a 0.0635-mm beryllium entrance window. The counter was filled with a gas mixture of Ar, CO$_2$ and He in the pressure ratio of 0.9 : 0.095 : 0.005 to a total pressure of 1 atm. Cylindrical aluminium collimators defined a field of view of 12.5° FWHM for the counter. The telescope was mounted with its look direction along the spin axis of the satellite. The details of the payload are being published elsewhere[1].

Observations on GX17+2 and GX9+9 were made by the proportional counter telescope in the thirty-first orbit, before the spacecraft was spin stabilised, when it was performing a slow spin around an axis different from the spin axis. Sighting of GX17+2 occurred at 110 d 8 h 9 min 8 s (UT), followed by that of GX9+9. The identifications of

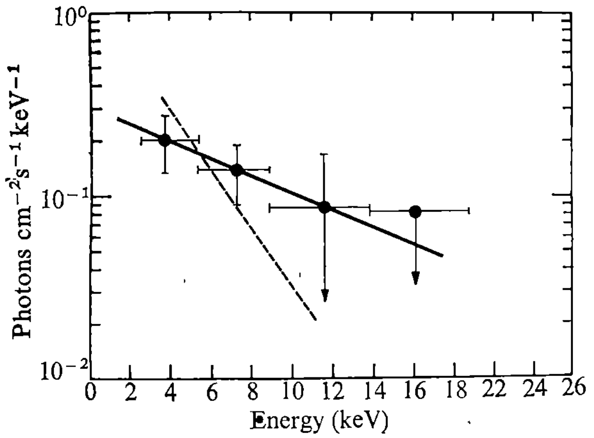
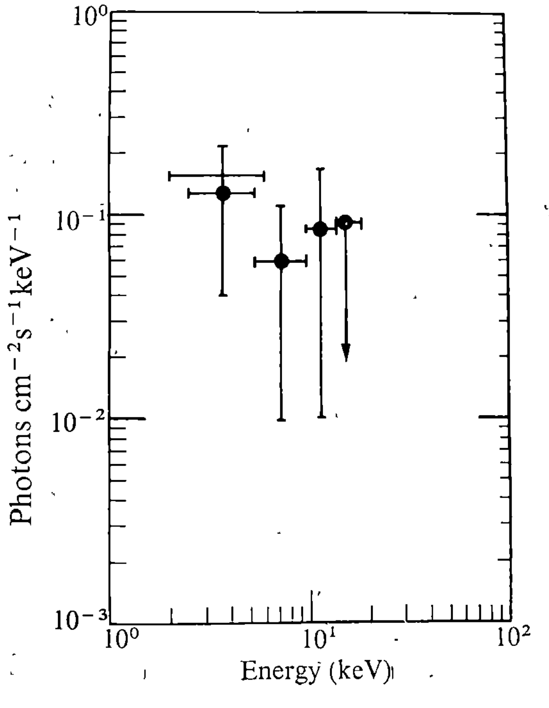


Fig. 1 Spectrum of GX17+2 obtained from Aryabhata observations. The dashed line represents the estimated temperature from the Lawrence Livermore group (42×10^6 K). The solid line represents our temperature (108×10^6 K).

GX17+2 and GX9+9 were based on the available positional data on these sources[2–4]. Data were obtained in four pulse height intervals over the energy range of 2.5–18.75 keV. The contributions of the two sources to the observed counting rates were derived after making allowance for the background effects as deduced from the examination of the nature of counting-rate profiles and their known geomagnetic dependence. Further, in the case of GX17+2, it was necessary to take into account contributory effects from GX+13.5 which was partially in the field of view for part of the observation time. These corrections were based on the available data[2] on this source, and the effect so estimated was ~ 20%. The photon spectra for GX17+2 and GX9+9 were derived after correcting for the efficiency of the counter and the telescopic response of the detector. The spectra so deduced for these sources are shown in Figs 1 and 2.

The spectrum of GX17+2 can be fitted to an exponential function in the present case, and yields a characteristic kT value of 9.31 ± 0.3 keV. If the X radiation arises from bremsstrahlung in a hot plasma, the equivalent temperature of the emitting body was $(108\pm4)\times10^6$ K at the time of observation.

Fig. 2 Spectrum of GX9+9 obtained from Aryabhata observations. ——, Uhuru; ●, Aryabhata.



GX17+2 is one of the relatively intense X-ray sources in the Scorpius–Sagittarius region. Observations on this source[5–7] had provided information primarily on its position rather than detailed emission features. The first set of detailed observations[8] were made by Uhuru detectors in late 1970 and early 1971 and revealed the variable character of X-ray emission besides establishing the exponential nature of the spectrum. Rocket-borne observations by the Lawrence Livermore Laboratory (LLL) group (R. W. Hill *et al.*, unpublished) have provided confirmation of the thermal nature of its emission. It seems that the source exhibits variations over a wide range of temperatures from 25×10^6 to 155×10^6 K. A closer analysis of the temperature–emission flux relationship in the 2–7 keV energy range leads, however, to no definite correlation between these two parameters. Further, when we consider all the existing data on this source, no definite conclusion can be drawn about the existence of a two-state emission character for this source as suggested by Tananbaum *et al.*[8]. The source seems to be similar in its properties to Sco X-1 as deduced from the comparison between radio and X-ray observations[9], but the lack of optical data on the source is a serious constraint to further analysis.

The spectral distribution of GX9+9 seems to be governed by a power law function with an exponent value of ~ 1.2, and is suggestive of a non-thermal mechanism for X-ray emission. In the energy interval of 2.5–10 keV, the integrated energy flux is $(0.57\pm0.3)\times10^8$ erg cm^{-2} s^{-1}. The flux obtained by Bradt *et al.*[3] in a rocket flight in 1967 was 0.25×10^{-8} erg cm^{-2} s^{-1} in the energy range 1.5–6 keV, whereas the same group[4] reported a flux of $\sim0.77\times10^{-8}$ erg cm^{-2} s^{-1} in the 1.5–8 keV energy interval from a subsequent observation in 1968. Comparison of the energy fluxes obtained from our observation and the two earlier rocket observations, after normalising to the same energy interval of 1.5–8 keV, leads to the suggestion that X-ray emission from GX9+9 is variable.

This work was supported by the Department of Space, Government of India. The launching of Aryabhata was made possible through the joint cooperation between the Indian Space Research Organisation and the Academy of Sciences, USSR.

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Received January 5; accepted January 30, 1976.

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Reliability of sunspots as tracers of solar surface rotation

NEW methods of measuring solar rotation have produced significantly different rotation speeds to those derived from sunspots[1,2]. Spectroscopic measurements of Doppler displacements give a so-called plasma rotation velocity of 13.76° d^{-1} (sidereal) at the solar equator[3] as against 14.38 ° d^{-1} (sidereal) from sunspot proper motion velocity[4,5]. Other techniques of measuring solar rotation lead to differences in the differential

Table 1 Sunspot properties and derived solar rotation velocities

	Spot SPO3703	Spot SPO3707	Spot SPO3710
Central meridian passage (UT)	Oct. 7.3 1975	Nov. 10.6 1975	Nov. 19.9 1975
Heliographic latitude	+32.5 °	+3.5 °	−7.0 °
Number of days observed during passage	13	9	11
Proper motion rotation (synodic) (degree d^{-1})	12.66±0.10	13.36±0.10	13.12±0.10
Proper motion rotation (sidereal) (degree d^{-1})	13.65±0.10	14.35±0.10	14.11±0.10
Plasma rotation (sidereal) (degree d^{-1})	12.47±0.15	13.16±0.20	13.61±0.20
Velocity difference (proper motion−plasma) (m s^{-1})	+140±20	+170±30	+60±30

rotation from a strong variation of the solar rotation with latitude for sunspots[4] and photospheric plasma[3] to hardly any variation at all for coronal holes[6]. Our understanding of these differences is very poor. It is therefore appropriate to obtain additional accurate solar rotation measurements with as many different techniques as possible and to check the validity of the assumption that the proper motion of tracers, like sunspots, correspond to the actual motion of matter on the solar surface. We report here the result of plasma rotation velocity measurements inside sunspot umbrae and their relation to the rotation velocities derived from spot proper motions. We find that plasmas inside and outside the spots rotate at similar rates, and conclude that sunspots make poor tracers.

The spectrographic measurements consist of sequences of spectra of the sunspot umbrae in the neutral titanium line at 571.39 nm. This line has been selected because of the following favourable properties[7]: (1) it has no Zeeman splitting; this makes it possible to locate its wavelength very precisely, independent of instrument polarisation properties: (2) it is strongly enhanced in sunspot umbrae with respect to the surrounding penumbra and photosphere; this changes in apparent lineshifts resulting from stray light from the atmosphere instrument and seeing: (3) it falls in a window between the numerous absorption lines introduced in the spectrum by an iodine absorption tube. The sunspot is imaged through this absorption tube, which is placed just in front of the spectograph slit. The numerous weak but very sharp iodine absorption lines provide a very stable wavelength reference for the Ti I line[8]. Since the iodine absorption spectrum is observed simultaneously with the sunspot spectrum (using light originating in the spot), effects arising from different spectrograph conditions, which often limit the accuracy when using emission reference lines, are eliminated. The spectra were recorded on Kodak SO-392 film with a dispersion of 96.9 mm nm^{-1} with an exposure time of ~ 2 min. The Sacramento Peak Observatory 16″ coronagraph was used to image the Sun, on the scale of ~ 8″ mm^{-1}.

Fig. 1 Difference in line-of-sight velocity for observed plasma velocity ($V_D{}^0$) and as derived from the sunspot proper motion ($V_D{}^{pm}$). The zero point for $V_D{}^0$ has not yet been determined, and is therefore not indicated. One division on the ordinate corresponds to 100 m s^{-1}. Error bars correspond to 1 s.d. The curvature of the data for the SPO3710 spot is probably the result of the third term in equation (1) indicating an upflow of ~125 m s^{-1} in the umbra.

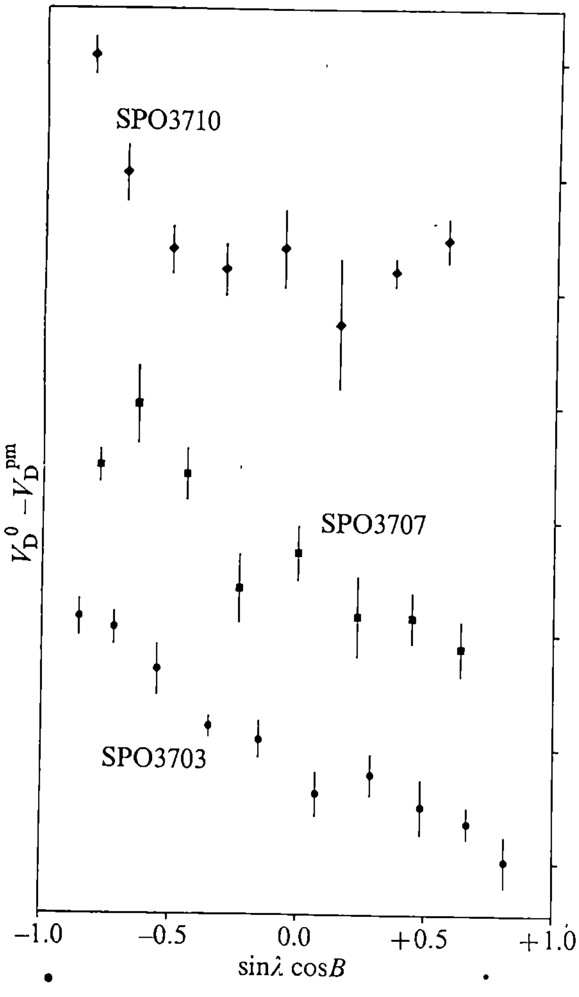

Table 1 lists the three sunspots for which rather complete coverage was obtained while the spot moved from the solar E–W limb. Two spots belong to the old cycle, one to the new. For each of the spots 60–100 spectra were obtained and analysed. The Ti I line could be located with respect to the nearby iodine lines with an accuracy 0.1 pm, corresponding to a Doppler velocity of ~ 50 m s^{-1}. Observations of the proper motion of the sunspots were made at the Sacramento Peak Observatory and at the NOAA Space Environmental Forecasting Center in Boulder. Table 1 also lists the synodic and sidereal rotation velocities derived from these proper motions. The latitude drift of the spots was very minor, $\lesssim 2$ ° during the spot passage across the solar disk (or $\leqslant 20$ m s^{-1}). After correction for the rotation and orbital velocities of the Earth the line of sight velocity V_D for a point on the Sun with heliographic longitude λ and latitude β may be shown to equal

$$V_D = V_\lambda \sin\lambda \cos B + V_\beta \cos\theta' - V_h \cos\theta \qquad (1)$$

where V_D is positive for a velocity away from the observer, (V_λ, V_β, V_h) are the velocity components on the Sun in positive longitude, latitude and height directions, B is the heliographic latitude of the centre of the solar disk, θ is the angle between the observed point on the Sun and the Earth as seen from the centre of the Sun and θ′ is the same as θ, but for a point in the opposite hemisphere at the same longitude, λ, but 90 ° away from the observed point. The last two terms in equation (1) are symmetrical around the central meridian (λ = 0), whereas the rotation term is not. This allows the determination of the rotation to be quite independent of any other velocity field. Figure 1 displays the difference in the observed Doppler velocity $V_D{}^0$ and the velocity $V_D{}^{pm}$ as predicted by the spot proper motions (and equation (1)) as a function of sinλ cos$B$. It is quite obvious that the observed plasma rotation velocity is significantly less than the spot proper motion rotation velocity. Table 1 lists the resulting plasma rotation velocities (degree d^{-1}) and the velocity differences from the proper motion values (m s^{-1}).

The difference between the two rotation rates is quite surprising since it is generally believed that the magnetic fields on the Sun are 'frozen-in' the plasma so that the two rotation rates should have been the same. The difference also shows that spots are poor tracers for measuring solar surface motions. The faster rotation of the spots (found to be close to the average sunspot values[4]) must be the results of subsurface effects, perhaps indicating an increase of rotation rate with depth. The slower plasma rotation velocities for sunspots makes the spot plasma rotate at similar rates as the plasma outside sunspots[3]. It should be noted here that P. Foukal (unpublished) found the solar surface plasma rotation velocity to increase with magnetic field strength in the plasma. This effect obviously does not persist for the very high field strengths found in sunspots. Future work will include comparison of spot and non-spot plasma rotation rates, analysis of the data for V_β and V_h velocity components and observations of (V_λ, V_β, V_h) for a number of sunspots within the same sunspot group.

I thank L. B. Gilliam, E. Coleman and E. Johansen for the observations described here, F. Reesly from NOAA, Boulder, for measuring the sunspot coordinates and W. J. Wagner for comments on the manuscript.

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Received December 15, 1975; accepted January 30, 1976.

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Physical argument and hypothesis for Sun–weather relationships

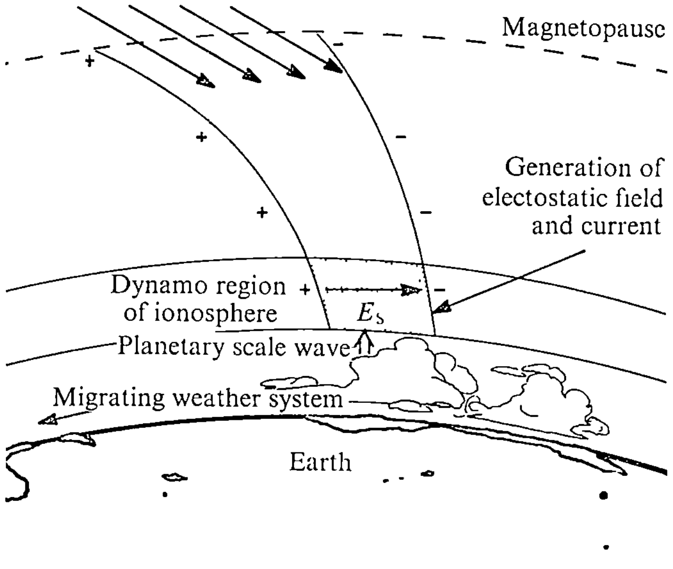
A GREAT deal of attention has been focused on the subject of Sun–weather relationships[1,2]. There is a clear need for physical processes and hypotheses to be brought forward and tested in this area. I propose here two theoretical schemes to account for some of the observed correlations; one is a physical argument suggested earlier[3], and expanded here, that the weather may generate electrical effects in the ionosphere (which may in turn affect the magnetopause), and the other is an hypothesis that the total emissivity of the Sun (both particles and electromagnetic radiation) remains constant. I investigate the possible consequences of this hypothesis.

First, the physical argument. There is strong evidence that weather systems in the lower atmosphere can launch energy into the ionosphere and there generate winds on a planetary scale[4]. In the dynamo region of the ionosphere the winds can generate electric currents and therefore magnetic disturbance directly[5]. Moreover, new electrostatic fields can be generated by the wind, which can be up to one order of magnitude greater than the wind dynamo fields[5]: their magnitude can be as great as 50 mV m^{-1} at the ionosphere. When there is good conductivity along the geomagnetic field, these fields can be mapped throughout the magnetosphere. If these new electrostatic fields occur on magnetic field lines connected to the magnetopause, solar wind plasma can be moved by the $\mathbf{E}\times\mathbf{B}$ drift into the magnetosphere[3]. The plasma so caught in the magnetosphere can be a source of energy for subsequent magnetic disturbance of direct solar wind origin. It should be unnecessary to state that there is continual interplay in this fashion between winds in the ionospheric dynamo region and the magnetopause, no matter what their origin. A field of 50 mV m^{-1} at the ionosphere could cause a field of $\sim$ 1.5 mV m^{-1} at the magnetopause because of the reduction in strength of the magnetic field there. This electric field is comparable in order of magnitude with that measured near the magnetopause by Aggson (see ref. 6). To drift solar wind plasma across the magnetopause one must have a component of electric field perpendicular to **B** and parallel to the magnetopause. Even if the angle between the electric field transmitted from the ionosphere and the normal to the magnetopause were as high as 85°, the drift speed of solar wind plasma across the magnetopause (where the magnetic field is assumed to be of strength $\sim$ 50 nT) would still be 2.6 km s^{-1}. Given a solar wind[7] of density 10 cm^{-3} and speed of 300 km s^{-1} and an area of capture of (30 Earth radii)^2, an input of energy to the magnetosphere of 7×10^{17} erg s^{-1} would be produced. This is comparable with the consumption of energy in the thermosphere from moderate magnetic disturbance[8,9].

In giving a physical interpretation to the correlations between magnetic disturbance and the weather in the lower atmosphere[10], one must further understand the migration path and the time constant of the weather systems. The complex wave communication time from the lower atmosphere to the very magnetopause may only be of the order of hours, but the time constant of weather systems of the order of days. It is entirely possible, then, that a primitive weather system while in polar regions could initiate magnetic disturbance in the above manner (see Fig. 1). This system could then migrate to middle latitude where it would seem to have followed, rather than initiated, the magnetic disturbance. This does not rule out the possibility that some (at this stage unknown) mechanism may allow magnetic disturbance to initiate some weather systems; on the other hand, the physical system propounded here could provide feedback to such an unknown mechanism. To test the foregoing theory, studies of correlations of magnetic disturbance and polar weather systems on or near magnetic field lines that connect to the magnetopause[11] should be performed.

We now present a new hypothesis. Note first that the solar 'constant' and the energy of emission of the solar wind near the plane of the ecliptic are similar when expressed in mass units through Einstein's well known relationship $E = mc^2$. The solar constant is[12] $\sim$ 1.9 calorie min^{-1} cm^{-2} $\sim$ 1.48×10^{-15} gcm^{-2}s^{-1}. The mean solar wind near the plane of the ecliptic at 1 AU from the sun has a density of 8 proton–electron pairs cm^{-3} and a radial speed of 320 km s^{-1}. This represents a mass flux of 4.3×10^{-16} gm cm^{-2} s^{-1}. The kinetic energies of the protons and electrons are not included in this estimate because they make only a small contribution. Of course helium and more massive ions may make up a substantial contribution to the mass flux also[7]. If the number density of helium reaches 25%, its mass flux then equals that of protons. A solar wind with ten proton–electron pairs per cm^3 and two helium ions per cm^3 and

Fig. 1 Modulation of solar wind capture by the weather



a speed of 633 km s^{-1} would equal, in mass flux, the solar 'constant'. It is possible that there is interplay between these two energy components at some fundamental level in the Sun.

Now the total energy (S) of emission of the sun may be written in mass units as

$$S = \int_0^{2\pi}\int_0^{\pi}\left[\int_0^{\infty}\frac{\varepsilon(\lambda)\mathrm{d}\lambda}{C^2}+\sum n_s m_s v+\frac{v}{8\pi C^2}[B^2+E^2+K]\right]\times$$

$$\times R^2 \sin\theta\mathrm{d}\theta\mathrm{d}\varphi$$

where $\varepsilon(\lambda)$ is the electromagnetic energy flux Å^{-1}; n_s, m_s, v the number density, mass and radial speed of a species s of particle; **B** and **E** the d.c. magnetic and electric field connected with the solar wind; K is the kinetic energy density associated with the thermal and organised motions of the solar wind particles; and R is radial distance from the centre of the Sun.

The most important of the terms of S in interplanetary space are those involving $\varepsilon(\lambda)$ and n_s, m_s, v. The theory of the solar wind[13] demonstrates the interaction of the parameters n_s, v and K in the lower reaches of the solar corona, where thermal energy density is converted to kinetic energy of expansion. There may be interplay between the terms involving $\varepsilon(\lambda)$ and those involving n_s, m_s and v at some levels in the Sun's outer layers perhaps somewhere in the chromosphere. I hypothesise that variability in S may be considerably less than variability of the component terms of S. The test of the possible constancy of the fundamental parameter S should be an added reason for an 'out-of-the-ecliptic-mission'.

This hypothesis has application to the modulation of the solar corona and to Sun–weather relationships and in particular to that involving interplanetary magnetic field sector structure[14].

On the first of these questions we note that extreme ultraviolet emission from the Sun may modulate the solar wind speed, as indicated below. In passing we note that the kinetic energy density flux of the solar wind ($\frac{1}{2}n_s\, m_s\, v^3$) is of the same order of magnitude as the combined extreme ultraviolet and ultraviolet flux at 1 AU from the Sun, that is, in the range 0.1–1 erg cm^{-2} s^{-1} (refs 7 and 15). There may be coupling between these fluxes. Indeed, recent X-ray images of the Sun show a relationship in space between depressions of X-ray intensity in the 3–32 Å and 44–54 Å band and increases in solar wind speed. Moreover, the depressions in X-ray intensities are related spatially to magnetic field structures on the Sun, being located between lines of polarity reversals[16]. We also note that magnetic structure in interplanetary space is mappable, to a good degree, with present knowledge, back to the Sun[17]. I suggest that the correlation of some weather systems and interplanetary magnetic field structure is traceable in physical origin back to the physical processes involved in the interplay of energy of solar wind at its origin, the magnetic field there, and the accompanying electromagnetic radiation. In terms of the hypothesis about S, this would mean that the weather modification associated with interplanetary sector structure is caused by a modulation of electromagnetic radiation (not necessarily restricted to the XUV range) emission from the outer layers of the Sun in specific regions in which the solar wind velocity and magnetic field structure are also altered. The possible occurrence of geomagnetic disturbance at an intermediate stage may be incidental and the weather modification can be traced more directly to the modulation of electromagnetic radiation at the Sun. Since it is now feasible technologically to look at a wide range of solar emissions with satellites, measurement of the comprehensive parameter S should be made, because it may be more basic to the understanding of Sun–weather relationships than the conventional solar constant. In the meantime it would be fruitful to search for a correlation in time of the conventional solar 'constant' and interplanetary sector structure. It should be noted that a correlation of time variations of intensities in the ultraviolet part of the solar spectrum with sector boundary crossings has already been noted[18].

I thank the NRC (USA) for the award of a Senior Postdoctoral Research Fellowship, Goddard Space Flight Center for hospitality, and La Trobe University, Australia for leave.

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Received October 3, 1975; accepted February 6, 1976

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Early lunar magnetism

WE have applied a new method[1] for palaeointensity (ancient magnetic field) to three subsamples of a single, 1-m homogeneous clast (72215) from a recrystallised boulder of lunar breccia. Samples from the clast have been dated by ^{40}Ar–^{39}Ar (ref. 2), Rb–Sr (ref. 3) and U–Pb (ref. 4) methods to yield a date of 4.0×19^9 yr as the age of boulder assembly. The new method[1] has two desirable characteristics; first, its reliance on simulating the natural remanent magnetisation (NRM) directly with a thermoremanent magnetisation (TRM); second, it includes a built-in monitoring device which detects thermally-induced sample degradation (oxidation, sintering and annealing), a common occurrence in lunar samples. We therefore provide here the first credible evidence that the strength of the ambient magnetic field at the Taurus–Littrow region of the Moon must have been about 0.4 oersted, 4.0×10^9 yr BP.

Of the two main models for the origin of the lunar magnetic field—the iron-core dynamo[5] and the uniformly magnetised lunar silicate core[6,7]—the former more easily explains our observation of an average magnetic field of 0.4 oersted during early lunar history. But in view of the similarity between the size of this field and that deduced[8–10] from carbonaceous chondrites 4.4×10^9 yr old, we draw attention to a relatively little considered possibility: that such large fields were characteristic of, and emanated from, a pre-main sequence, T-Tauri stage Sun.

The details of our experiments will be published elsewhere. The NRM of lunar rock 72215 was found to be very stable[11], and the remanences carried by the desirably high coercivity

Table 1 Palaeointensity values from 72215

Subsample no.	N	H (oersted)	s.d. (02)	Range (oersted)
72215,56	7	0.55	0.10	0.74–0.44
,46	7	0.41	0.17	0.60–0.21
,93	8	0.28	0.07	0.37–0.18

range of 100–800 oersted were used to calculate palaeointensities. Table 1 lists three palaeointensity values, which are roughly 0.3, 0.4, and 0.5 oersted respectively. Within one standard deviation, the values 0.3 and 0.4 obtained from subsamples 72215,46 and 72215,93 do not differ significantly from each other. The value of roughly 0.5 oersted obtained from 72215,56 is, however, different and may be expected to represent the lunar palaeointensity during the formation of the boulder (4.0×10^9 yr BP); the mean palaeointensity of 0.35 oersted obtained from the other two subsamples may pertain to an older time. We prefer however, to adopt a more conservative attitude: that of calculating an average palaeointensity of 0.4 oersted from all the three subsamples, and assigning them all an age of approximately 4.0×10^9 yr. In the present experiment, for the first time, three subsamples from one rock (72215) have been heated to beyond their respective maximum blocking temperatures, and anhysteretic permanent magnetisation (ARM) curves before and after heating have shown that little change in the magnetic carriers took place during the heating. The deduced palaeointensities are based on comparisons of laboratory TRMs in a known field, with the NRMs acquired as TRMs during the assembly of the boulder.

Leaving aside other palaeointensity measurements involving approximate methods such as simulating the TRM of the lunar samples with an ARM (refs 12–14) or induced permanent magnetisation (IRM) (ref. 15), there are four determinations involving complete heatings to the highest blocking temperatures of lunar samples with approximate ages of 4.0×10^9 yr. Our measurements from the three subsamples reported here indicate a value of 0.4 oersted. The other reliable measurement[16], taken from a single piece of 62235,53 (dated at 3.9×10^9 yr) yielded a value of 1.2 oersted. A replotting of those data to meet criticisms[15] does not change the observed value by more than 20%. Thus, we need to find a source for a rather large, ancient lunar magnetic field at $\sim4\times10^9$ yr BP. The magnitude of this field could be as low as 0.4 oersted (our measurements) or as high as 1.2 oersted (Collinson *et al.*[16]). In either case, the required fields are approximately 10^4 times greater than present-day interplanetary or solar flare fields.

Sonnett and Runcorn[17] have outlined a rather ingenious mechanism, which could generate a lunar, iron-core dynamo, using inductive heating as a heat source, in a small accreting Moon. It meets one of the difficulties[15,18] of an operating dynamo in the young lunar core. The present state of knowledge in theoretical magnetohydrodynamics is, however, such that we cannot convincingly say whether such a small dynamo would be able to produce fields on the order of 1 oersted (see ref. 14). In our opinion, however, if we have to choose between a lunar core dynamo and the field of a permanently magnetised, lunar silicate core, once exposed to an even larger primordial field, the first alternative seems more preferable. Strangway and Sharpe[7] have calculated that a primordial field strength of 10 oersted would have been needed to produce that degree of magnetisation in the silicate core (with 3% by weight of iron) which would lead to an ambient palaeointensity of 0.02 oersted at the lunar surface. Such a low palaeointensity is indicated[19] by an apparently reliable thermal determination on a young (3.3×10^9 yr) lunar breccia, 15498,36. But using the latest value[20] of iron content for lunar interior (9% by weight) and our lunar palaeointensity of 0.4 oersted at 4.0×10^9 yr, the updated value of the postulated primordial solar field has to be as large as 75 oersted. Faced with this dilemma of generating 0.4 oersted (or as much as 1.2 oersted) at the lunar surface by invoking either a primordial field of 75 oersted uniform over 10^3 km, or a marginally plausible, central iron-core dynamo that can generate 0.4 oersted at the lunar surface, we prefer the latter model.

A third solution may be even more acceptable than those two. First, we draw attention to the apparent coincidence between the range of 0.4 to 1.2 oersted for a lunar surface field at 4.0×10^9 yr BP, and the reported[8–10] range of 0.2–1.1 oersted for palaeointensities determined from carbonaceous chondrites approximately 4.4×10^9 yr old. The latter values are $\sim10^4$ times greater than the present interplanetary field, and if such a field were attributable to a pre-main sequence, T-Tauri stage Sun (with no sector structure of the solar field), it could indeed be the same large, early Solar System field postulated[21] to explain the smaller solar angular momentum of that time. Could the lunar surface at 4.0×10^9 yr BP not, in fact, have recorded the same early field? Alternatively, the ambient solar field could have decayed from 1 oersted at 4.4×10^9 yr BP to, say, 0.1 oersted at 4.0×10^9 yr BP. The apparently higher (0.4–1.2 oersted) ambient field at the lunar surface would then be attributable to an amplification of the 0.1-oersted field by 'dusty' plasma dynamos operating on the lunar surface, and generated by the intense collisions[22,23] which seem to have gone on at the lunar surface till 4.0×10^9 yr BP (ref. 24).

There is one apparently strong objection to this hypothesis. Lunar rocks aged $4.0–3.2\times10^9$ yr old have been found to be magnetised. Thus, it is commonly assumed that the same source of the lunar surface field had to operate continually over the given time range. We do not agree with that contention, and point to a possible recorded drastic decrease in the lunar field after 4.0×10^9 yr BP. The palaeointensity[19] of 0.02 oersted at 3.3×10^9 yr BP is the single reliable, thermally determined value, at present available for those younger times. Future palaeointensity studies may confirm such a low (by two orders of magnitude) value, and thus point to quite a different source for the lunar field, such as the magnetohydrodynamic amplification of a 0.002-oersted ambient solar field, or even the presence of an older underlying crust, magnetised at an earlier time. Regarding the latter, it should be recalled that station magnetometer readings as large as 0.003 oersted produced locally by the magnetised crust were measured by the Apollo-16 astronauts[25]. So far as the present work is concerned, we believe to have presented credible evidence for a large magnetic field during early lunar history which could most easily come from a pre-main sequence T-Tauri Sun. In as much as we do not have to invoke an external field that was both dipolar and uniform over the Moon as a whole, our conclusion here does not invalidate the suggestion[26] that the global lunar magnetic anomaly record could not be attributable to an external, uniform, dipolar field.

We thank NASA for financial support.

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Received October 13, 1975; accepted February 3, 1976.

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Radiocarbon evidence for deglaciation in north-western Himalaya, India

SINCE the publication of the first post-glacial pollen diagram from Toshmaidan (74°31′E, 33°56′N, ~ 3,120 m OD) in the Kashmir Valley by one of the authors[1], the age of the pollen sequence has been a bone of contention[2–4]. The organic deposits overlie a sequence of bluish-grey buttery clay and crushed rock, and were suggested[1] to date from the last deglaciation. The pollen sequence was divided into eight stages, and showed a cold–warm–cold climatic sequence, which was similar to previous findings on post-glacial climate[5]. Here we present radiocarbon dates on these deposits, and these confirm our previous work.

In early June 1973, an expedition to Kashmir was organised to obtain radiocarbon dates of the inorganic–organic interface, as well as the sedimentary sequence in the mire, at Toshmaidan, and a position stratigraphically close to the original point was selected for collecting samples. The samples dated were subjected to pollen analysis for correlation with the original pollen diagram from the site.

The results of the radiometric dating are shown in Table 1. All but one sample were dated at between ~ 9,500–15,000 b.p., showing that the sedimentation of the organic deposit at Toshmaidan started long before the Neolithic period. The samples which were pollen analysed showed that all samples predating 9,500 b.p. belonged to the time of the first four pollen stages (*a–d*) (Table 1). The next sample above, however, correlated with the seventh stage (*g*) of the pollen sequence, and gave a date of 2,790 ± 160 b.p. As none of the samples showed any similarities with stages *e* and *f* it was suggested that there was an erosional break between samples PRL-**2(B)** (2,790 ± 160 b.p.) and PRL-**3** (9,650 ± 245 b.p.) which correlated with stages *g* and *d* respectively. The gap of nearly 7,000 yr between the radiocarbon dates from the above two samples (Table 1) confirmed the presence of the break in sequence. Because of this disconformity in the sequence it is not possible to calculate the rates of sedimentation in the whole sedimentary profile. This also means that pollen stages *e* and *f* in Singh's pollen diagram cannot yet be dated accurately. Nevertheless the dates for these stages cannot be outside the range set by PRL-**2(B)** (2,790 ± 160 b.p.) and PRL-**3** (9,650 ± 245 b.p.), and therefore the stages could still represent broadly the mid-postglacial time to which they had been assigned in ref. 1. Similarly stage *h*, the topmost stage, can now be considered to postdate 2,790 ± 160 b.p. Radiocarbon dates from samples comparable with stages *a–c* fall between ~ 14,000 and 15,000 b p. and with stage *d* between 9,500 and 11,000 b.p.

Terminal dates for the pollen stages all remain to be calibrated but it may not be possible to do so accurately without a detailed pollen analysis and fresh radiocarbon dating from the site.

The most significant point to emerge from the present study has been the evidence of deglaciation at Toshmaidan which is shown to have been in progress at ~ 3,120 m OD in Kashmir, ~ 14,000–15,000 yr b.p. This date, from the contact between inorganic and organic sediments, is in agreement with the dates generally associated with deglaciation following the last glacial period in several parts of the world[6]. The regional significance of the date for deglaciation from Toshmaidan will become clear as more dates become available from other parts of the Himalayan region. In the global context, the date of deglaciation from Kashmir broadly falls in line with dates obtained from similar elevations in New Guinea[7] and Ruwenzori, Uganda[8] in the tropics. There are, however, no available sites providing information on age of deglaciation situated at similar latitude and altitude as Toshmaidan, and, as a result, exact parallels have not yet been found.

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Table 1 Radiocarbon data from Toshmaidan

PRL number	Serial number	Depth (cm)	Material	Sieve fraction (μm)	Pollen stage (ref. 1)	Radiocarbon date (yr b.p.) (λ taken as 5,568 yr)
2(B)	II	15–35	Peat	<420	*g*	2,790 ± 160
3	III	50–70	Peat	<420	*d*	9,650 ± 245
4(B)	IV	75–90	Peat	<420	*d*	$10{,}005^{+340}_{-380}$
5	V	125–140	Peat	<420	*d*	$11{,}360^{+585}_{-600}$
7	VII	205–220	Fine organic mud	—	*c*	$13{,}980^{+520}_{-565}$
9	IX	280–295	Clay mud	—	*c*	$15{,}250^{+760}_{-820}$
10	X	317–327	Clay mud	—	*a–b*	$14{,}760^{+1015}_{-925}$
12	XII	337–350	Blue-grey lacustrine clay	—	*a–b*	$13{,}850^{+900}_{-785}$

Eastern Greenland basalts and their supposed plume origin

IT has been suggested[1,2] that the eastern Greenland flood basalts had their origin in plume activity centred about a triple junction of which Kangerdlugssuaq (Fig. 1) is the failed arm. The suggestion is reviewed here in light of the petrological characteristics and spatial distribution of the basalts.

The main outcrop of lavas is on the Blossville coast in the 450 km between Kangerdlugssuaq and Scoresby Sund, though scattered, less well known outcrops are found along the 800 km of coast between Kialineq and Shannon Isle. The lavas were extruded during an early stage of the opening of the North Atlantic from sources on the seaward side of the present outcrops. They thin inland from the coast, where estimates[3] of their thickness in the neighbourhood of Ryberg Fjord indicate a maximum of 9 km Though Nielsen[4] has suggested that thickness estimates may be overgenerous because of a failure to take into account faulting, we do not believe that the figures are significantly in error. The base of the pile is submarine and the upper 6 km comprises dominantly, but not exclusively, subaerial flows. Allowing for several kilometres of thinning between Kangerdlugssuaq and Scoresby Sund, and taking into account basalts on the coastal shelf, the present volume along this 450 km of coast is estimated as approximately 6×10^5 km^3.

Palaeontological dating using marine microfossils of the submarine base of the lava pile in the Kangerdlugssuaq–Ryberg Fjord area[3] and marine horizons in the top of the pile at Kap Dalton[5], shows that the effusive activity occurred in latest Palaeocene time over a very restricted time interval, the best estimate of which is approximately 3 Myr. It may be that the activity took place within one geomagnetic reversal[3], and if that is so then even 3 Myr may be an overestimate.

Using the best available estimate of 3 Myr, however, the volume of basalts erupted each year in this one area of flood basalts is 0.2 km^3, well in excess of the estimated maximum discharge of the Icelandic plume model[6], and as shown in the reconstruction of the North Atlantic (ref. 7, and Fig. 2) the actual extent of the flood basalts must have been much greater. Particularly intense activity is indicated and there is no evidence in the lava pile of regional evolution with time from alkaline to tholeiitic (of the sort found, for example, at the Afar triple junction[12]).

Analyses of subaerial basalts from a typical section in the area of Wiedemann Fjord (Table 1) show a content of large ionic lithopile elements (LIL) characteristic of 'hot spot' basalts, as distinct from relatively LIL-depleted mid-ocean ridge basalts[8]. Published analyses of subaerial basalts from the area of Scoresby Sund[9], are comparable. Schilling[10,11] has suggested that 'hot spot' basalts typically have a lanthanum-samarium enrichment factor $(\text{La/Sm}_{\text{EF}})>1$ and he has presented analyses from Scoresby Sund which show this feature[11]. The Wiedemann Fjord analyses likewise show a $\text{La/Sm}_{\text{EF}}>1$, and also the light rare earth pattern typical of 'hot spot' basalts (Fig. 3). Schilling and Noe-Nygaard[11] have shown that the Lower and Middle Series basalts of the Faeroe Islands also have $\text{La/Sm}_{\text{EF}}>1$ and are comparable with the Scoresby Sund basalts, though, as they pointed out, Scoresby Sund in the Atlantic reconstruction is significantly distant from the Faeroes.

Tholeiitic basalts with these characteristics are typically found at oceanic hot spots such as Iceland, or occur late in the process of crustal thinning, as in the Afar region[12,13]. They show no evidence of continental crustal influence in their chemistry and satisfy the criteria of volume and compositional homogeneity indicative of basalts generated directly by mantle fusion. The Greenland basalts overlie continental crust but their chemistry and the low pressure regime necessary for their voluminous generation by direct mantle melting[14,15] indicate that they were erupted from the embryonic ocean forming offshore from the present outcrop after thinning of the continental crust. The evidence is that this part of Greenland never attained any significant elevation above sea level before or during the effusive activity[3,5], and it is not difficult to envisage the lavas spreading on to the continental crust and thinning away from their source area.

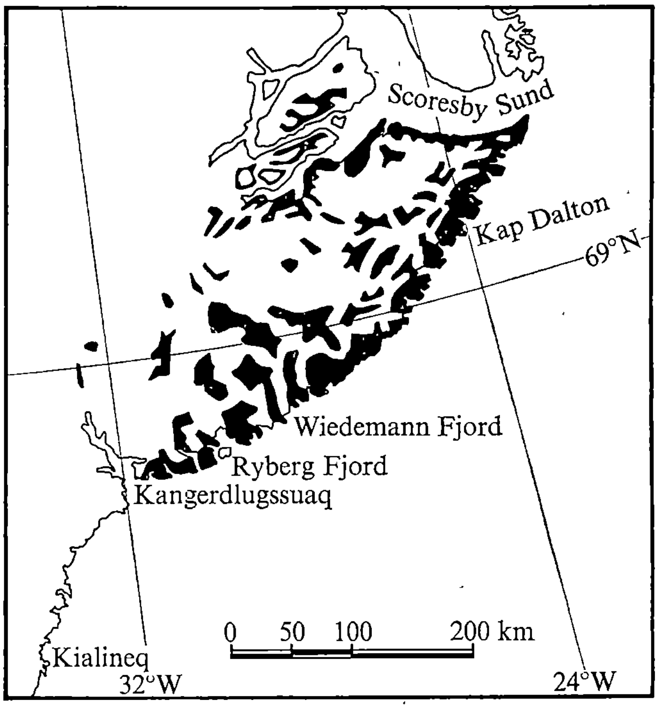

Fig. 1 Localities and basalt distribution between Kangerdlugssuaq and Scoresby Sund.

The original plume hypothesis of Morgan[16] postulated plumes of the order of 150 km diameter rising from the deep mantle. As developed by Schilling[10] and Vogt[17] for Iceland, a much larger plume of geochemically undepleted mantle, 200 km in radius, rises beneath Iceland and provides material which spreads preferentially away beneath the mid-ocean ridge. Geochemical anomalies of decreasing magnitude are found up to 400 km away from Iceland[10] (that is, up to three times the plume radius from its centre), beyond which normal (relatively depleted) mid-ocean ridge lavas become dominant. Schilling[10] attributed the systematic variation in rare earth and minor element concentrations, which he documented along the Reykjanes Ridge axis, to the mixing of relatively undepleted (plume) and depleted (asthenosphere) materials or their partial melt products (though later developments[18,19] have shown that the variations may be step-like rather than gradational). Flow rates away from the plume beneath the mid-ocean ridge have

Fig. 2 Bott and Watts'[7] reconstruction of the North Atlantic, showing position of Faeroe Isles and probable distribution of basalts 55 Myr BP.

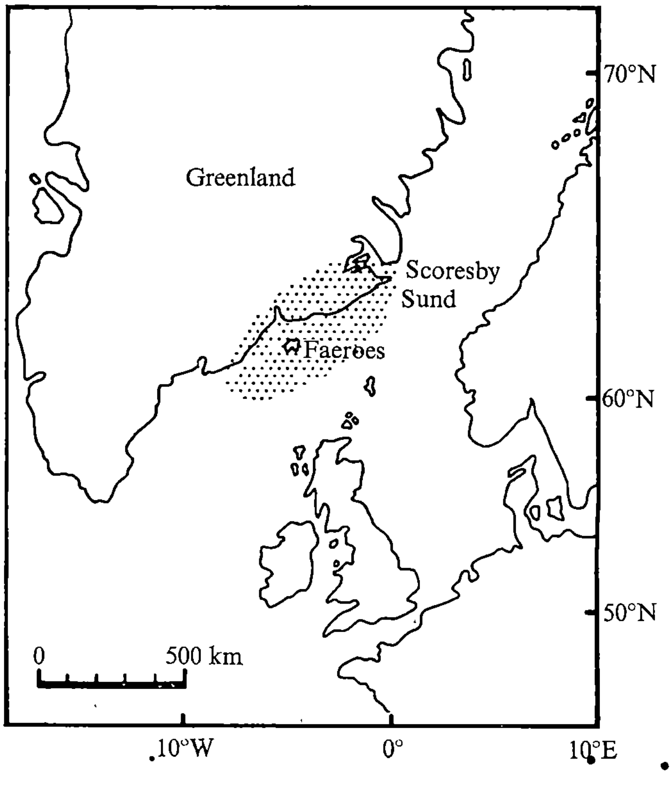

Table 1 Analyses of subaerial basalts from a typical section near Wiedemann Fjord

	Wiedemann Fjord*	Scoresby Sund†
SiO_2	47.51±0.39	49 0
Al_2O_3	13.74±0.34	13.8
TiO_2	2.21±0.23	1.9
Fe_2O_3	4.35±0.65	6.2
FeO	8.84±0.85	8.2
MgO	7.07±0.38	6 6
CaO	11.37±0.72	11.1
Na_2O	2.26±0.31	2.3
K_2O	0.29±0.19	0 3
MnO	0.17±0 06	0.2
P_2O_5	0.21±0.02	0.2
La/Sm_{EF}*	1.38 0.15	

*Mean ± s.d. of 11 basalts from Wiedemann Fjord (analysts V. A. Somogyi and R Kanaris-Sotirion).

*La/Sm_{EF} for 10 of these specimens (analyst J. E. Whitley).

†Average of 37 analyses from Scoresby Sund.

been estimated[17] at 10–20 cm yr^{-1}, and 5–7 Myr must have elapsed for the geochemical anomalies to reach the furthest distance from the plume at which they can be detected. If the plume model is applied to Greenland 55 Myr BP, then the distribution of the basalts would likewise indicate preferential movement of the plume material along the developing lithospheric weakness from which the lavas erupted. If a plume of the large 200-km radius and flow characteristics suggested for Iceland was centred about Kangerdlugssuaq, then several million years would be expected to elapse between the initiation of magmatic activity over the plume and the build-up of the voluminous extrusions actually found along the length of the coast. The evidence, however, suggests that chemically similar, undepleted magmas erupted in large volumes simultaneously between Kangerdlugssuaq and Scoresby Sund, the whole effusive activity occupying approximately a mere 3 Myr. On the plume hypothesis, an escalation in plume intensity or an unlikely increase in dimensions to something double that for Iceland would be necessary.

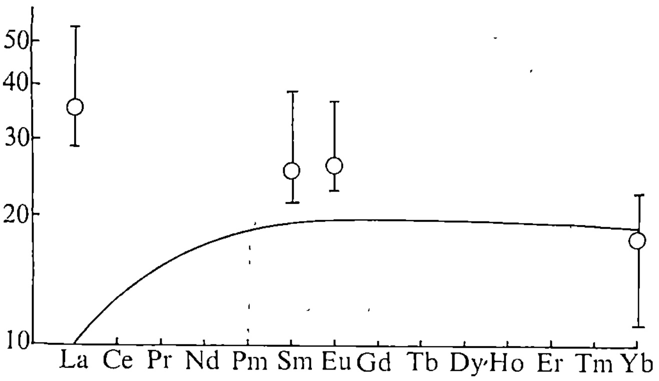

Fig. 3 Pattern of chondrite normalised, selected rare earths for Wiedemann Fjord basalts, showing average and spread (analyst J. E. Whitley). Lower line, chondrite normalised, mid-ocean ridge average pattern[22].

It is concluded that in their spatial and temporal characteristics the eastern Greenland flood basalts do not easily conform to the model of a narrow plume beneath continental crust. The rise of a discrete mantle blob, as opposed to a long lived plume (see ref. 11), a model which offers an explanation of sudden chemical changes with time, has similar difficulties of dimensions and timing when applied to the eastern Greenland–Faeroes situation. A convective system of wide lateral extent, or perhaps the linking of several lithothermal systems into a major convection system[20,21] seems preferable. Nevertheless the problem of separate magma sources for hot spot and mid-ocean ridge basalts, or the alternative of appealing to phlogopite stability and disequilibrium melting[15], still remains.

We thank N. J. Soper and C. Downie for discussions.

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Late-glacial and postglacial sedimentation in the Peris-Padarn Rock Basin, North Wales

BOREHOLES sunk in the Llyn Peris Trough, North Wales, have revealed considerable thicknesses of Quaternary deposits, including glacial lake rhythmites, sands and gravels, together with substantial amounts of organic lake mud and wood debris peats rich in pollen and macrofossils. Preliminary sedimentological and palaeontological analysis and radiometric dating indicates that the sedimentological record extends from the period of ice wastage in the valley heads into the Christian era.

The gravels, sands, silts and peats were deposited in a rock basin produced by glacial overdeepening of the Cambrian shales, slates and gritstones. The lowest lithostratigraphical unit consists of laminated lacustrine clay-silts which are locally sandy and gravelly They are glacilacustrine rhythmites predominantly of diatactic type and, so far, they have proved to be barren of pollen and macroscopic organic remains. They exceed a thickness of 27 m (borehole EP7: Fig. 1) and they rest on rock-head at about 35 m OD in the vicinity of borehole EP4. In thin section, both coarse and fine laminae can be seen to contain an appreciable amount of clay-sized material, the range of grain size in the coarser layers (1–10 μm) being about one order of magnitude larger than in the finer laminae. Grain counts of the relatively few clasts with long axes greater than 0.2 mm revealed a predominance of feldspars, muscovite, quartz with some grains of magnetite, chlorite and partly altered biotite The finer material in the coarser layers and the material in the finer layers was found to be dominantly of quartz with feldspars, muscovite and some chlorite. The finer laminae include a notable amount of very fine grained clay mineral. Analysis of this clay mineral component by X-ray diffraction showed a dominance of muscovite, chlorite and kaolinite, in addition to

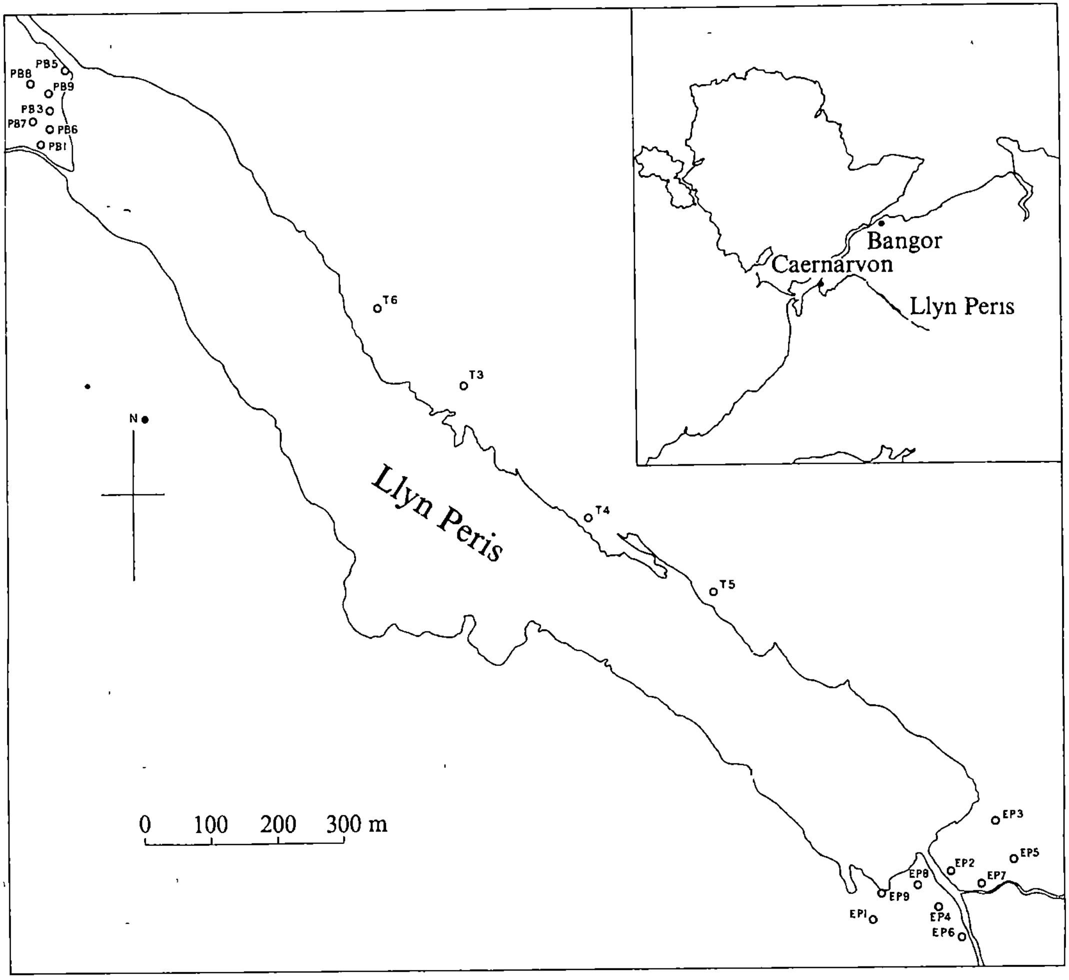

Fig. 1 Location of Llyn Peris and sites of boreholes.

quartz. The kaolinite has a high crystallinity index, indicating a clastic origin, attributable to the grinding action of ice. There is little amorphous clay and little difference in the clay mineralogy of the coarse and finer layers. The microfabric of the silts and clays is being investigated using the scanning electron microscope.

Although severely disturbed in local areas because of overburden from slate quarries, the rhythmites make up a regular series of plane beds with only occasional discontinuities caused by local penecontemporaneous erosion and minor faulting.

Varve counting methods have been applied to the total length of the rhythmites in borehole T3. The mean thickness of rhythmite sets (which are assumed to represent deposition over 1 yr, and thus to be true varves) is 12 mm (range 2–50 mm), the number of coarse and fine couplets within each varve varying from 1 to 8. Given that the rythmite sets are true varves, the results suggest that accumulation extended over a period of 738 yr, or 850 yr if an estimate is included for gaps in the core.

At the head of Llyn Peris and in the large fan at Pont-y-Bala separating Llyn Peris from Llyn Padarn, the rhythmites are overlain by beds of glacifluvial origin. These sediments range in size from very coarse cobble and pebble gravels to thin layers of fine and medium sand associated with the rhythmites and the overlying organic deposits. The constitution of the gravels is dominated by the local bedrock types, the slates and shales giving high average flatness values in all grades, and moderate to high roundness values. Clasts of medium gravel grade and larger show faceted forms. In the case of the grits and vein quartz that is probably attributable to glacial grinding. The medium sand grade, probably the most susceptible to edge rounding, is moderately to well rounded. The greatest thicknesses of sand and gravel are found in the large fan at Pont-y-Bala, which comprises interlayered sands, gravels, rhythmites and peats.

The sands and gravels are the products of periodically high discharges of meltwater streams which issued from the Peris Glacier above the lakehead and from waning cirque glaciers above Pont-y-Bala[1]. This gravel fan is of particular interest. As it is interbedded with rhythmites, it evidently began to accumulate well before the final vestiges of ice disappeared from the adjacent cirques and higher valley heads. The interbedding of the sand and gravels with peat indicates that the fan continued to grow well into the postglacial period. Thus, fan growth imposed a progressively rising water level on the Llyn Peris Basin.

Complete bores and bulk samples of the organic layers (earlier obtained by shell and auger rig) have been examined from sites at both ends of Llyn Peris. Macroscopic examination has revealed three distinct suites of organic deposits.

The Pont-y-Bala suite consists of discontinuous wood peat lenses, between 0.5 m and 2 m thick, interbedded with gravels. Borehole PB6 traversed three such lenses (Fig. 2). The wood, which is of *Betula/Alnus* type, is highly compressed and distorted. This feature, together with the high mineral content of the peat, suggests that the material has been rafted into the Pont-y-Bala fan from adjacent slopes. The East Peris suite from the head of the basin is much more impressive. Borings have revealed organic deposits up to 20 m thick buried by 10–12 m of sands and gravels. Material from the EP7 borehole at depths between 10 m and 30 m (Fig. 3) is highly variable, comprising organic lake muds, wood and debris peat with well preserved

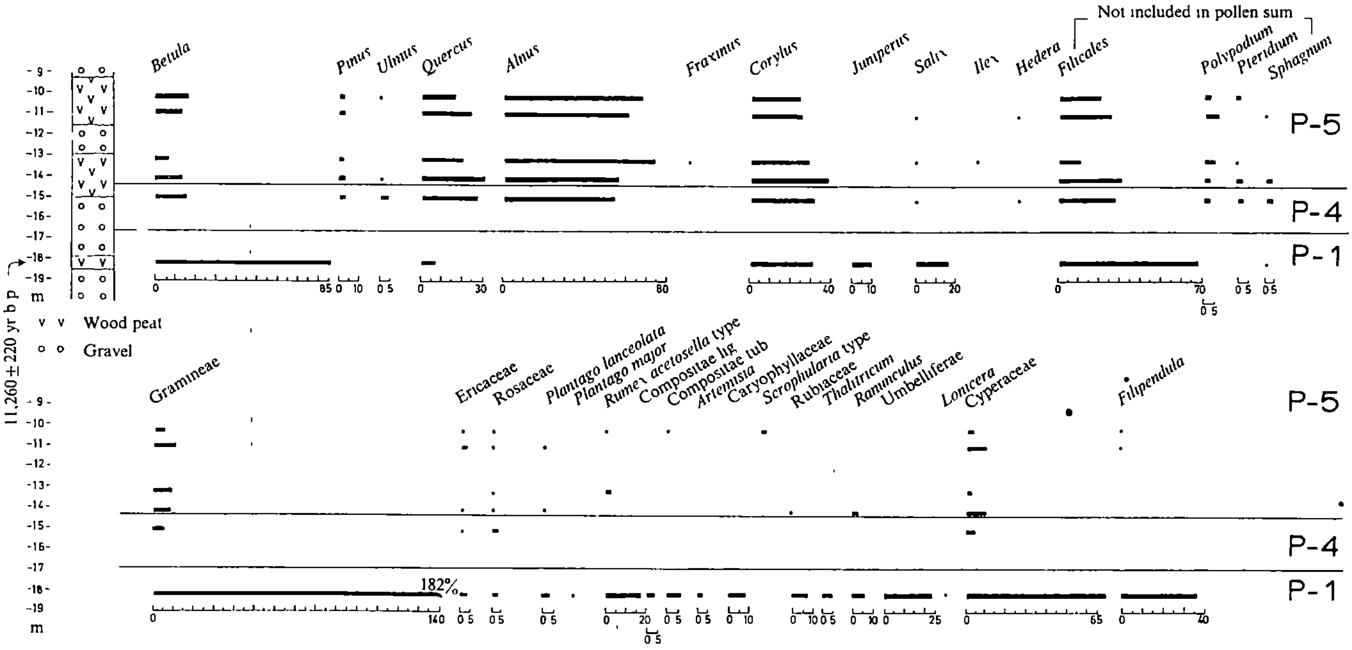

Fig. 2 Tree and non-tree pollen expressed as percentages of total tree pollen for PB6 at depths of 9–19 m

leaves, and sand and gravel partings. These deposits indicate accumulation *in situ* in a marginal lacustrine environment with a rapidly rising water table. The Central Peris suite of organic deposits is not discussed in detail here. This suite lies beneath Llyn Peris and beneath the Dinorwic slate tip, which has extended out into what was formerly the lake. They comprise fine-grained, dark-grey organic lake muds and silts, typical of a deep lacustrine environment.

Figures 2 and 3 show the pollen diagrams from boreholes PB6 and EP7. The sampling interval was determined by the availability of bulk samples from those cores. Six pollen assemblage zones, Peris 1–6, have been recognised, and their characteristics are summarised in Table 1. They are listed in what is assumed to be chronological order, although the sequence in PB6 is interrupted because of the discontinuous nature of the deposits.

The earliest assemblage, Peris 1 has a late Weichselian date of 11,260±200 yr b.p. (Table 1, B-555), and has similarities with the late-glacial floras described from Cwm Dwythwch and Nant Ffrancon[1].

The assemblage suggests a fairly open environment, as the proportions of pollen of light-demanding plants are high. It is likely that scrub of birch and willow was fairly widespread in the basin. If the theory of rafting is correct some of this material may have originated in the Cwm Dwythwch area, as this lies immediately above the Pont-y-Bala fan. Figure 3 shows the full sequence of pollen assemblage zones (Peris 2 to Peris 6), and these exhibit the standard changes which are associated with the develop-

Table 1 Characteristics of Peris pollen assemblage zones

Pollen assemblage zone	Representative samples	Characteristics	Affinities and dating†
Peris 1 *Betula/Filipendula/Rumex* assemblage	PB6 18.55 m	*Betula* >80% AP.* *Filipendula* >30% AP. *Rumex* >10% AP Gramineae >150% AP. AP* values relatively low. Wide variety herb pollens.	Late Weichselian 11,260±220 yr b.p.
Peris 2 *Betula/Pinus/Corylus* assemblage	EP7 29.5 m	*Betula* >50% AP. *Pinus* >30% AP. *Corylus* >100% AP. Herbaceous types well represented but reduced in variety compared with Peris 1.	Flandrian chronozone F I 9,400±200 yr b.p. (start zone)
Peris 3 *Pinus/Ulmus* assemblage	EP7 28.0–28.95 m	*Pinus* >40% AP. *Ulmus* >10% AP. *Betula* <30% AP. *Corylus* <60% AP. *Alnus* rising. Low representation of herbaceous types.	Flandrian chronozone F II
Peris 4 *Alnus/Quercus/Ulmus* assemblage	PB6 15.0 m EP7 23.75–28.0 m	*Alnus* >60% AP. *Quercus* >20% AP. *Ulmus* >5% AP. Low representation of herbaceous types	Flandrian chronozone F II
Peris 5 *Alnus/Quercus* assemblage	PB6 9.4–14.5 m EP7 15.45–22.8 m	*Alnus* >60% AP. *Quercus* >25% AP. *Ulmus* <2% AP. Low representation of herbaceous types. Representation of *Betula* increases at expense *Quercus* at top of zone.	Flandrian chronozone F III
Peris 6 Gramineae/*Plantago lanceolata* assemblage	EP7 13.72–10.6 m	Gramineae >75% AP. *Plantago lanceolata*> 5% AP. A wide variety of herb pollen types represented.	Flandrian chronozone F III 1,750±100 yr b.p. (end zone)

* AP, Arboreal pollen.

† Radiocarbon dates provided by Department of Geology, University of Birmingham.

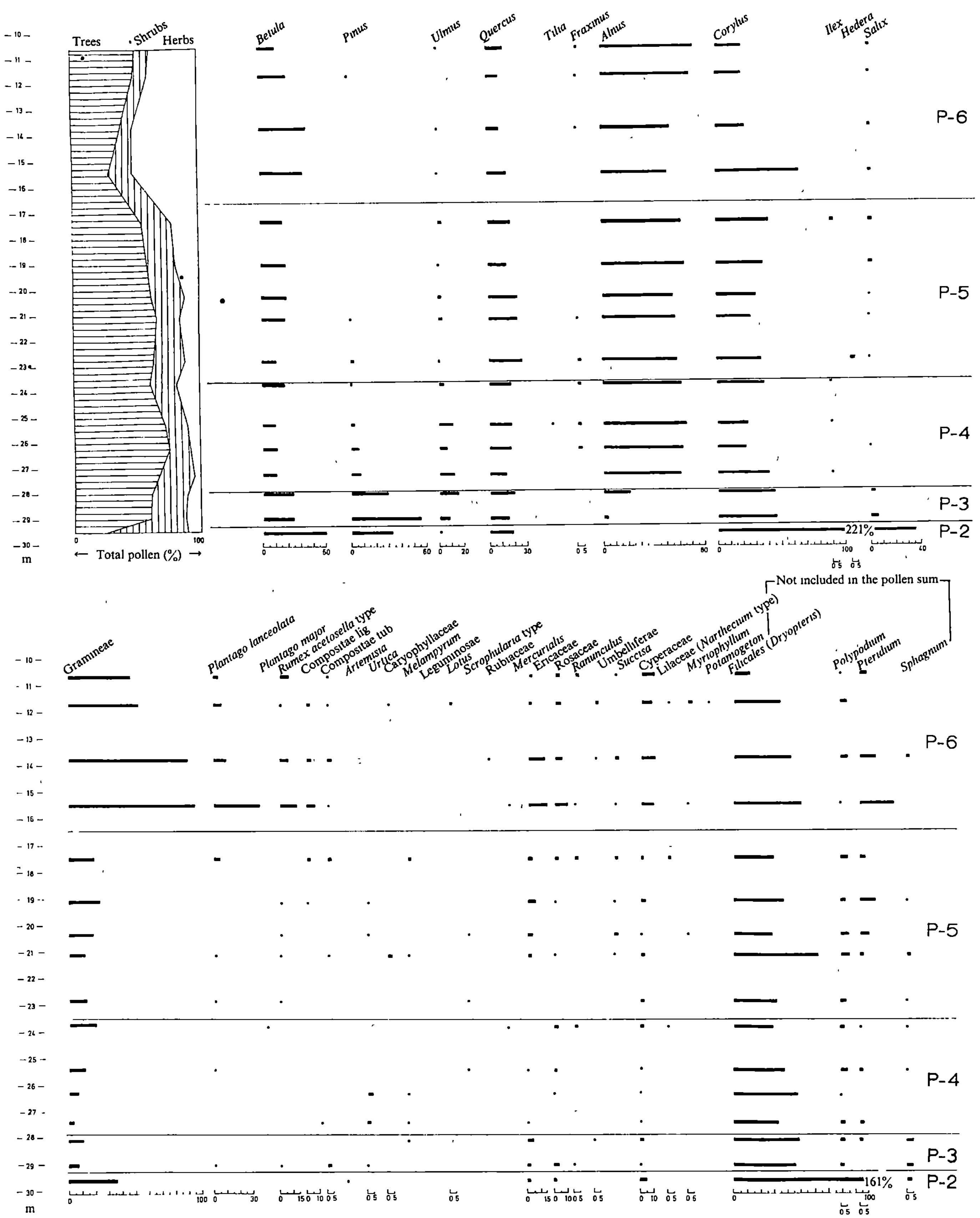

Fig. 3 Tree and non-tree pollen expressed as percentages of total tree pollen for EP7 at depths of 10–30 m.

ment of Flandrian vegetation in the British Isles. Material from Peris 2 has a radiocarbon date of 9,400±200 b.p. (Table 1, B-554). The high percentages of *Corylus* pollen typical of this assemblage occur at many sites in the British Isles in Flandrian chronozone Ib (ref. 2). The woodland canopy in the basin was becoming closed at this stage, although some open areas clearly persisted. Stands of pine existed, with birch and hazel scrub being widespread. Peris 3 is characterised by an increase in *Pinus* pollen at the expense of *Betula*. Pine must have come to dominate the woodlands of the basin in this zone, but oak and elm were spreading and alder was already present in the area.

Mixed oak forest became well established in the Peris-Padarn basin during Peris 4. The expansion of *Alnus* which marks the opening of this zone, is usually assumed to correlate with the FI/FII chronozone boundary around 7,000 yr b.p. The stratigraphical evidence suggests that dense alder carr grew at the eastern end of Llyn Peris at that time, probably extending all around the lake and partly up the slopes as the assemblage is also represented in the rafted material from Pont-y-Bala. The canopy seems to have been very dense with few herbaceous plants surviving. The *Ulmus* decline horizon, assumed to date from around 5,000 yr b.p. (ref. 3) has been used to separate the Peris 4 and Peris 5 assemblage zones. Apart from the decline in elm in the area the forest composition does not seem to have changed markedly. In contrast, the high proportions of pollen of Gramineae and herbs in Peris 6 are indicative of man's interference with the vegetation on a large scale. *Plantago lanceolata* is a herb particularly associated with pastoral farming activities, and it seems probable that the inception of this zone dates from the Bronze Age. Organic material from the top of the zone has a radiocarbon date of 1,750 ± 100 yr b.p. (200 AD: B-556).

The sequence of vegetation history follows the pattern already established for western Britain. Further detailed analyses of these and other cores from the area should allow the reconstruction of a complete picture of the ecological development of the basin. Particular attention will be paid to establishing the nature and scale of man's activities in this highland area.

Two features of the deposits of this basin are of outstanding interest. The first is the very great depth of peat, of the order of 20 m, which has accumulated in 9,500 yr at the East Peris site. This can only be accounted for in terms of a very rapidly rising water table which must have been governed by the growth of the alluvial fan at Pont-y-Bala. The second feature of particular interest is the 10 m of gravel which buries these East Peris deposits—gravel which has accumulated in the basin since 200 AD. Certainly, catastrophic events must have been involved in its deposition. It seems that disruption of the vegetation cover on the steep hillsides triggered this wave of sedimentation. Whether this was the product of climatic oscillation or early man is still to be determined.

We thank the Central Electricity Generating Board and Binnie and Partners, Consulting Engineers for allowing access to the core material on which this study is based.

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Received December 22, 1975; accepted February 9, 1976.

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Relationships of Middle and Upper Pleistocene hominids from sub-Saharan Africa

IT has long been common to refer to the Broken Hill (Kabwe) and other later Pleistocene African hominids as representive of a "Neaderthaloid" population in the sub-Sahara In the first account of the Rhodesian skull, Smith Woodward[1] notes similarity to the "Neanderthal or Mousterian race", and this theme has been repeated, with qualifications concerning the extent to which African Neanderthals actually resemble their European relatives[2-4]. Brose and Wolpoff[5] lump all African and Eurasian specimens under the heading "Neanderthal". Following Mourant[6], a few recent studies[7,8] have cast doubt on the interpretation; these are based on measurement and use distance statistics which show separation between African and European materials but provide little information about important anatomical differences involved. I report here morphological evidence of the distinctiveness of Broken Hill and its relationships to other African remains. Examination of original fossils from Hopefield (Elandsfontein), Florisbad and the Omo as well as Broken Hill reveals a pattern quite unlike that of Neanderthal crania from Europe.

The Rhodesian skull roughly resembles Neanderthals and other archaic *Homo sapiens*, in that the facial skeleton is large below the massively developed supraorbital torus, the braincase low, and the occiput rather more sharply angulated than in modern man Broken Hill, however, is extreme in supraorbital development, and the torus is absolutely thicker than in comparable later Pleistocene material (Table 1). Frontal flattening is marked, in spite of some keeling in the midline. The frontal is also relatively narrow as noted by Mourant[6], so that torus exaggeration produces lateral flare of the anterior temporal lines, and the temporal fossae are deep. These characteristics are not matched in European specimens, and Neanderthals generally have a supratoral sulcus (ophryonic groove) backed by a prominent, more bulbous frontal region "Classic" Neanderthal crania also have extensive flattening of the parietal vault near bregma, while there is lateral swelling of the braincase towards asterion With some variation, there is rearward extension of the occiput to form a bun-like structure. Although the Rhodesian cranium has limited parietal flattening along the posterior course of the sagittal suture, characteristic asterionic bulging of the vault is not apparent, and this part of the braincase seems poorly filled by comparison. Greatest breadth is in the supramastoid region rather than higher on the parietals as in most Neanderthal specimens. Viewed from the side, the occiput is nearly vertical above a strongly developed torus, so that actual bunning of the bone is slight. This has been overemphasised in discussions in which Broken Hill is labelled an African Neanderthal.

Striking aspects of the Rhodesian face are its biorbital width and extreme length, especially below the nasal opening. But this face has been shown to be less projecting at nasion and subspinale than various Neanderthals measured[9]. Inspection reveals relative flatness of the maxillary frontal processes and the walls below the orbit, whereas in European crania there is obvious retraction of the zygomatic arch relative to the facial midline. Broken Hill has some alveolar prognathism, but only here does protrusion begin to approach that recorded for Neanderthal remains.

There are such departures from "classic" Neanderthal morphology in other African skeletons, though there has been little effort to document them systematically. Parallels between the Rhodesian and Hopefield fossils have been emphasised[2,10] and these are most obvious in the frontal region. Torus form is certainly similar. The Hopefield parietals are slightly more rounded laterally, but maximum breadth is still low, near the mastoids (which are missing). The base is also broken away, but the form of the occiput seems to be like that of Broken Hill, even if a torus is not so well developed. A fossil with similar morphology is Eyasi I, though this represents a smaller individual, perhaps a female. A cast in the British Museum (Natural History) shows the supraorbital torus to be less robust than in the southern skulls, but in overall appearance Eyasi I is strikingly like Hopefield.

The Omo skeletons[11] now provide more information from East Africa, and Omo II has a few traits not seen in Broken Hill. The Omo II frontal bone is substantially broader than

Upper Circular Road
Calcutta

Table 1 Measurements of Middle and Upper Pleistocene hominid crania from East and South Africa

Measurement (mm)	Broken Hill	Hopefield	Omo II	Omo I	Florisbad*	Olduvai Hominid 9
Cranial length	205	200‡				206
Torus thickness†						
central	23	21			15	19
lateral	16	15			12	14
Basion-nasion	108					119‡
Basibregmatic height	127					110‡
Max. cranial breadth	145		147			150
Biauricular breadth	140§		132			135
Max. frontal breadth	118	114	121		132§	
Min. frontal breadth	99	102	108		116§	88
Biorbital chord	125				124§	
Malar height	29				24	
Frontal chord	120	116‡			120	
Frontal angle (degrees)	18	20‡			23	
Parietal chord	111	109	119			
Occipital chord	87		106	101		
Occipital angle (degrees)	40‡		34	35		

All measurements were taken on original specimens. Techniques and definitions are discussed ref. 24.

*The Dreyer reconstruction of Florisbad has been disassembled, and the measurements listed are those which can be obtained from the separate frontal and facial parts. Until a new reconstruction is attempted, dimensions of the orbit and nasal opening cannot be reliably reported.

†Supraorbital torus development is difficult to quantify, and my measurements do not correspond exactly with others reported (for example, ref. 2). One reading is taken in the centre of the orbital margin, though this is not always the point of maximum thickness. The second measurement is taken close to the frontomalar suture, to gauge the extent of lateral attenuation of the torus.

‡These measurements make use of reconstructed landmarks.

§Values obtained by doubling a reading to the midline.

the Rhodesian specimen and quite flat, lacking a supratoral sulcus. The supraorbital margin is intact for only about 4 cm from the left frontomalar suture, and here torus development is slight; however, thickening of the bone towards the centre of the orbit suggests more massive construction in the middle of the face. Outcurving of the anterior temporal lines is not marked, and the temporal fossae are not deep as in Broken Hill and Hopefield. But the skulls are similar in side view, and the vertex lies well forward near bregma. Behind this point, the Omo profile drops evenly away for about half the parietal arc length and then dips again, though the resulting step-like contour is not as obvious as in Broken Hill. There is slight keeling of the parietals in the midline, and there is none of the lambdoid flattening characteristic of European Neanderthals. Instead the parietal wall falls sharply from the temporal line to the mastoid, so that the sides of the cranium are almost concave above the massive mastoids and supramastoid crests, and there is no bulging of the parietals at asterion. Occipital curvature is comparable with that of Broken Hill and Hopefield, but the facial skeleton is missing.

Omo I is less complete but has been termed more modern in appearance. Certainly the back of the occiput is higher relative to the reduced nuchal plane, and the entire bone seems less flexed than that of Omo II. Prominent but localised raised areas of muscle insertion on the nuchal surface are misleading, however, and absolute differences in curvature are small (Table 1) The vault is higher and has a more rounded parietal profile from behind Several frontal fragments cannot be fitted to the parietals but suggest a flattened forehead not unlike that of Omo II.

Given these features of the Omo skulls, several hypotheses regarding their affinities deserve examination. Leakey[12] views the Omo material as separate from "Neanderthaloids" such as Broken Hill and Hopefield This may be reasonable if only the frontal is considered, but obvious similarities in total pattern between Omo II and the southern remains cannot be ignored. Other workers see a major division within the Omo assemblage and prefer to set only Omo I apart, as more modern than Omo II as well as Broken Hill Statistical results[8,13] which seem to support this are based on few measurements or on reconstructions, however, and it is questionable whether a firm case for two lineages in East Africa can be made on the evidence available. An alternative suggestion[8] that Omo I is sampled from a more recent segment of the lineage also containing Omo II cannot be verified geologically[14].

While there is uncertainty about Omo I, the remaining sub-Saharan crania including Omo II are not only long and low in outline but distinctive in more specific ways. Frontal flatness (whether or not associated with extreme supraorbital development), an anteriorly placed vertex coupled with a step-like sagittal contour, near vertical parietal walls above a massive and broad cranial base, and an angulated occiput are all features of a braincase poorly filled by modern standards. The rear of the skull, which lacks asterionic expansion and shows no real occipital bun, is not like that of European Neanderthals, nor is the Broken Hill face, which is broad and devoid of projection in its upper parts. Surely these African hominids are better referred to as archaic than as Neanderthal-like[15], and this term also describes the mandibles usually assigned to the later Pleistocene[16]. A formal subspecific designation as *Homo sapiens rhodesiensis* now seems justified on morphological as well as geographic grounds. Recent revisions of dating support this view and suggest that the African material is substantially earlier than the Würm age appropriate for European specimens Klein[17] argues for a Middle or earliest Upper Pleistocene date at Broken Hill, and the bulk of the Hopefield fauna is also likely to be Middle Pleistocene[18] An aspartic acid racemisation age for a femoral fragment in the Broken Hill assemblage is reported as 110,000 yr (ref. 19), and there is a Th/U date of 130,000 yr for member I of the Kibish Formation in which the Omo skulls were found[20].

If a later Middle Pleistocene age can be accepted for this material, comparisons with other earlier African fossils are more convincing. Leakey[21] notes resemblances of Broken Hill and Hopefield to hominid 9 from bed II at Olduvai, but this important find has never been described fully. Hominid 9 is about equal in length to the Rhodesian skull and exceeds it in maximum breadth across the base by 5 mm It is also lower and more rugged in general morphology, and inion lies well above the horizontal plane The occiput is more strongly curved, and the extensive nuchal surface merges evenly on to the broad posterior faces of the pyramidal mastoid processes. That this heavy flattened cranium is easily distinguished from Broken Hill is not surprising, if the Olduvai hominid is roughly 0.9–1.0 Myr in age[22]. But there are points of definite similarity, particularly in the frontal region. Torus development and postorbital constriction are marked on hominid 9, though the straight brow is not quite so thick as that of Rhodesian man There is also general resemblance in occipital proportions to the later skulls, especially Omo II, although this braincase is more rounded at the back and bears modern looking mastoids. Since the functional significance of such craniofacial likeness is uncertain, the issue of relatedness must be approached with caution. But, the still archaic skulls of *Homo sapiens rhodesiensis* can be regarded as somewhat expanded, higher versions of the Olduvai vault, and the populations sampled at Broken Hill and in the Omo are probably evolved from local groups of early Mid-Pleistocene *Homo erectus.*

Where some individuals (for example, Omo I) depart from the archaic morphology of Broken Hill, there is a suggestion of continuity with more recent hominids. The Florisbad frontal exceeds even that of Omo II in breadth, and brow ridges must be more comparable in size with the (incomplete) Omo structures than with those of Rhodesian man Glabellar protrusion is similar to Omo I. The Florisbad forehead is, however, not as evenly flattened anteriorly, and in torus form and temporal fossa depth this specimen rather recalls the architecture of Broken Hill and Hopefield. Even if Florisbad is correctly dated at 39,000 yr (ref. 23), it is far from fully modern in construction. Facial fragments should provide more information, and fresh study will tie Florisbad more firmly into the succession of later Pleistocene African populations.

I thank B. Hendey, R. E. Leakey, M. D. Leakey, J. J. Oberholzer, C. Stringer and M. Tessema for access to fossil material. M. H. Day commented on a draft of the manuscript, and the work was supported by a grant from the National Science Foundation.

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A spatial approach to insect population dynamics

THERE are two aspects of the regulation of animal numbers: first, how the numbers in a population are controlled around a mean level over a number of generations and second, how this mean level is determined. Studies on the dynamics of insect populations leading to the construction of life tables are frequently confined to a particular location where the fecundity and mortality of successive generations are estimated[1-3], and this approach emphasises the first aspect. Varley[1] appreciated the value of obtaining permanent traces of the insects as they passed from one instar to another (galls, emergence holes, exuviae), since such direct counts of the numbers entering an instar avoided the time-consuming and inaccurate process of integrating successive estimates of population size[4]. The traces are analogous to a natural accumulative trap. Beaver[5] and Danks[6] used this 'trace' method for bark beetles and various solitary Aculeata. Freeman[7] extended the trace method to accumulated generations at each of several sites, an approach which emphasises the second aspect of population dynamics.

Our studies in Jamaica on the population dynamics of solitary aculeates commenced with the wasp *Sceliphron assimile* Dahlbom[7-9]. This sphecid builds mud cells, in each of which a single egg is laid[7]. Thus the number of cells constructed by a female constitutes a long-lasting record of her fecundity. Instars which die within cells constitute a similar record of mortality, whereas emergence holes simply measure adults emerged. Moreover, the predators, parasites and competitors can be identified by traces of their entry into or exit from the cells, or by the cast exuviae or faecal pellets within them. The traces thus represent data accumulated for many successive generations[8].

In temperate countries the alternation of summer and winter tends to regulate the synchrony of generations, but in the tropics this is frequently not so and the concept of generation loses much of its value. Although *S. assimile* breeds at a reduced rate between January and March and some prepupae diapause, during the rest of the year about five to six overlapping generations occur. Disused cells accumulate with very small losses for at least seven years or some 40 such generations. Cells belonging to different generations or years cannot be accurately separated. Thus their study and dissection at any site provide data which although not generation(time)-specific are location(space or place)-specific, since we could not expect the pattern of fecundity and mortality relating to so many generations to be very different from any other equal or longer period. Note, however, that the variation of fecundity, mortality and migration with time cannot be assessed.

It is a relatively short task to dissect a large sample of cells from a particular locality, and from this to construct a retrospective place-specific life table. Many localities can be investigated and compared, and thus the spatial pattern in population dynamics can be more simply explored since the temporal one is suppressed. Also, life tables from many localities can be summed to produce one which is a species-specific character for an entire population and therefore of similar status to a human life table for a particular nation.

The major problem in this work is to relate the density of the disused cells at any site to that of the living animals on which mortality factors have been operating. The former are naturally the sum of the latter, but errors would arise if cells had been accruing at one site for a longer period than at another, or if the disappearance rate of disused cells was greater at one site than at another. The numbers of living and disused cells of *S. assimile* were correlated[8] ($r = +0.96$), but errors can be further reduced by sampling at comparable sites—for example, rejecting sites where cells have been accruing for only a year or two. Although specifically unaccountable errors must be present, we estimated[10] in *Pachodynerus nasidens* (Latr.) their magnitude to be about ±20%, which is relatively small compared with the range of density of disused cells (about 1–1,000 m^2).

Within the limitations of our method, density-dependent mortality with respect to place is well established in our tropical aculeates. *Melittobia ?hawaiiensis* (Eulophidae), (formerly recorded as *M. chalybii* Ashmead) is the chief parasitoid of solitary Aculeata in Jamaica. The correlation coefficients of percentage mortality caused by it on the density of the following Aculeata were: *Sceliphron assimile*: $r=+0.84$, $P<0.0001$ (ref. 8); *Zeta abdominale* (Drury), (=*Eumenes colona* Saussure): $r=+0.72$; $P<0.01$ (ref. 11); *Pachodynerus nasidens*: $r=+0.52$, $P<0.01$ (ref. 10) In *Zeta abdominale* predation by the miltgrammine fly *Amobia floridensis* Townsend was again density dependent, $r=+0.60$, $P<0.02$ (ref. 11). Developmental mortality was high at places where these species were usually dense. Since time is not accountable we are unable to detect delayed density dependence[1].

Since so many generations are being considered, mortality and fecundity must balance unless there is strong evidence that the species is becoming more (or less) numerous and/or extending (or contracting) its range Even so they will tend to be equal. To analyse the situation further the k-value budget of Varley and Gradwell[12] which measures the $\log_{10}$ differences between successive population density estimates in a single generation, can be modified to suit the spatial approach. First, estimates of the density of any developmental instar are necessarily accrued so that the k values (here called $\hat{k}$ values) between them measure total mortality for a particular stage over several generations. Note that they are not mean k values ($\bar{k}$) since dense generations would over-contribute to them. Second, since over the period during which the cells are accruing, fecundity and mortality will be almost exactly equal, a cyclic budget returning to a constant value—say, at the egg stage—can be constructed:

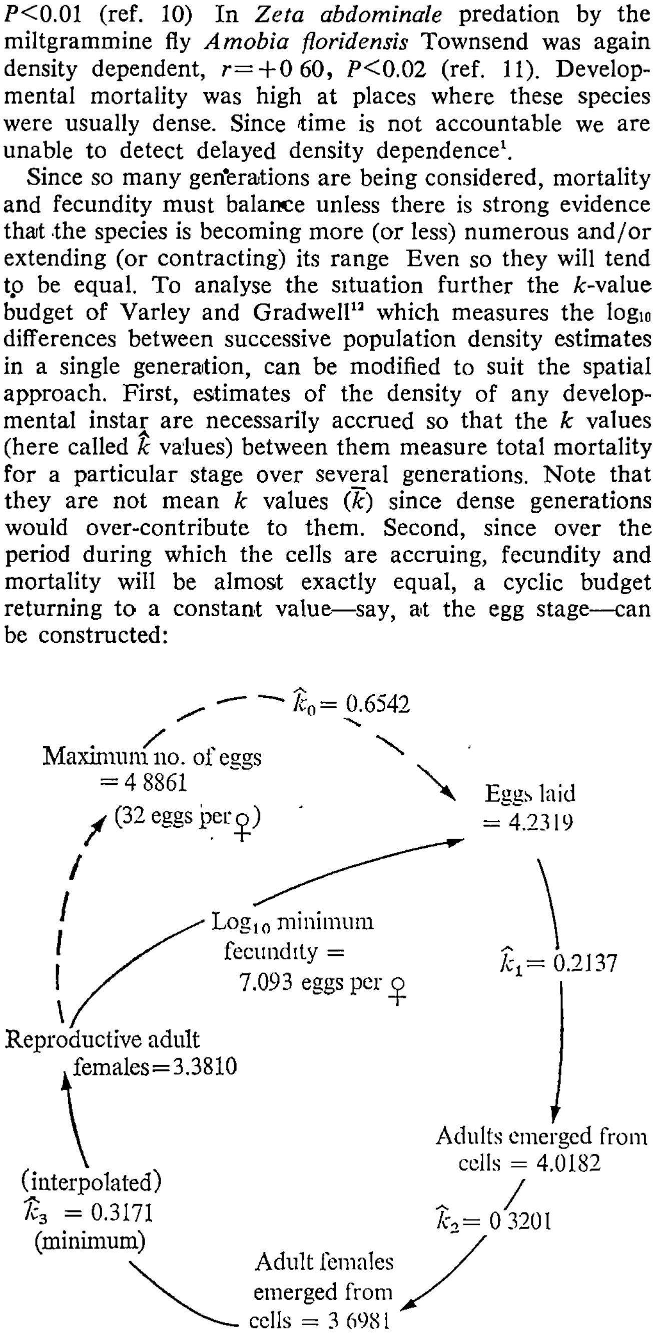
$\hat{k}_0 = 0.6542$
Maximum no. of eggs = 4 8861 (32 eggs per ♀)
Eggs laid = 4.2319
$\log_{10}$ minimum fecundity = 7.093 eggs per ♀
$\hat{k}_1 = 0.2137$
Reproductive adult females=3.3810
Adults emerged from cells = 4.0182
(interpolated) $\hat{k}_3 = 0.3171$ (minimum)
$\hat{k}_2 = 0.3201$
Adult females emerged from cells = 3 6981

Fig. 1 A cyclic budget for *S. assimile* in Jamaica (see text). $\hat{k}_0$, Loss of fecundity; $\hat{k}_1$, developmental mortality; $\hat{k}_2$, loss to males, $\hat{k}_3$, pre-reproductive mortality/migration of females.

Figure 1 traces the fate of 17,059 ($\log_{10}=4.2319$) eggs laid during some 40 generations The sex ratio was equivalent to about 47.8% females, and the remaining males are considered as a 'loss' to the species and therefore equivalent to a mortality factor. The pathway of maximum fecundity (broken line) would occur if all reproductive females laid the maximum number of eggs recorded for a single female. Minimum fecundity is the average number of cells per nest in Jamaica corrected for those cells in which no egg was laid[7]. Observations on marked females indicate that fecundity achieved was 10–15 eggs per reproductive female. $\hat{k}_0$ measures the maximum loss of fecundity due to the death of reproductive females before they have laid all their eggs. The budget has the important property that any missing $\hat{k}$ value (here $\hat{k}_3$) can be interpolated. Thus, if achieved fecundity was 12 5, $\hat{k}_3$ would rise to 0.5631 and $\hat{k}_0$ would fall to 0 4082.

Budgets for specific localities will contain an immigration–emigration factor and this will usually occur in the preproductive adult stage; but the one above is for Jamaica as a whole, and therefore we expect this factor to be small. Budgets for specific localities provide quantitative data on how population levels are achieved and their comparison may enable factors causing the specific population level to be identified, but they cannot reveal the mechanism of control Budgets for entire populations enable inferences about the life strategy of a particular species to be made.

I thank O. W. Richards, FRS, K. Ittyeipe, B. Y Johnston, D. B. Jayasingh, A. Raw, D. Rogers and C. A. Taffe for discussions and Dorothy Graham for assistance

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Received November 7, 1975, accepted January 20, 1976

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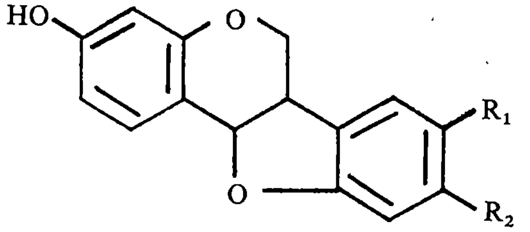
Phytoalexin induction as a new dynamic approach to the study of systematic relationships among higher plants

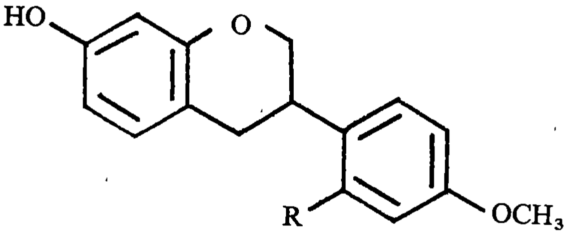
IT is well established that many higher plants respond to microbial invasion by the *de novo* production of organic substances called phytoalexins[1,2] These compounds are absent from healthy plants and are induced by the attacking microorganisms Although the role of phytoalexins in disease resistance is not yet entirely clear, considerable evidence suggests that they are of importance in the protection of higher plants from fungal colonisation Although few surveys have been attempted, there is clearly a taxonomic element in phytoalexin biosynthesis, in that different plant families accumulate chemically different types of compounds[3]. Thus, the Leguminosae in general produce isoflavonoids, the Solanaceae diterpenes, the Compositae polyacetylenes and so on[2]; anomalies are rare, for example, the furanoacetylene, wyerone acid, from *Vicia faba* (Leguminosae)[4] As lesser variations also occur within these families, there is the clear possibility of using phytoalexin induction as a tool in taxonomic studies. We report here the first successful application of this technique to the problems of classification at the generic and species level in the Leguminosae.

The first genus studied was *Trigonella*, a group of some 70 annual species, the best known member being the spice plant, *T. foenum-graecum* *Trigonella* is a taxonomically 'difficult' genus because it merges into the closely related *Medicago* and *Melilotus*[5]; indeed, some of its species have at one time or another been variously classified under these other two genera Young plants were grown from authenticated seed samples of 35 *Trigonella* species. Phytoalexins were obtained from detached leaves using the drop-diffusate technique[6]; conidial suspensions (about 5×10^4 spores ml^{-1}) of the non-pathogenic fungus, *Helminthosporium carbonum* in 0.05% aqueous Tween-20 were used as the phytoalexin inducer[7]. Control leaves were inoculated with deionised water containing Tween-20. After 48 h of incubation, the droplets (diffusate) into which the phytoalexin had diffused, were collected and the antifungal component(s) isolated and identified by direct comparison (thin-layer chromatography, ultraviolet and mass spectroscopy) with authentic samples. The fungitoxicity of compounds I, II, III and IV was determined in tests against the mycelial growth of *H. carbonum*.



I: $R_1=H$; $R_2=OCH_3$

II: $R_1=R_2=O—CH_2—O$



III: $R=OH$

IV: $R=OCH_3$

All 35 species produced one or more of five chemically related phytoalexins. Two of these were the known pterocarpans, medicarpin I and maackiain II while a third (compound TC-1) was provisionally identified as a new tri- or tetrahydroxy-pterocarpan. The others were the known isoflavans, vestitol III and sativan IV. All five compounds were absent from the control diffusates. Traces of three pterocarpan precursors, namely the isoflavone formononetin, the flavanone liquiritigenin and the chalcone isoliquiritigenin accompanied the above phytoalexins in a few species. The results of surveying all 35 species clearly indicate that *Trigonella* can be divided into three broad groups (Fig. 1); group 1, with medicarpin and maackiain; group 2, with medicarpin alone; and group 3, with medicarpin and vestitol. These groups were also divisible into several subgroups (Fig. 1). Thus, within the eight species of group 1, three produce medicarpin and maackiain in equal amounts, whereas the other five produce much more of the former than of the latter. Similarly in group 3, there is a division between the three species which synthesise medicarpin and vestitol and the eight which also produce sativan.

That phytoalexin studies allow *Trigonella* species to be placed within groups which are systematically meaningful is apparent from supporting morphological and chemical evidence. In particular, many species from group 2 show some morphological resemblance to *Melilotus*, a genus characterised by the production of medicarpin. Furthermore, a special chemical feature of *Melilotus*, namely the ability to release coumarin when tissue is macerated[8], is present in most (11 of 16) of the *Trigonella* species in this group (Fig. 1) but is otherwise absent from the genus. Also, the 11 species of group 3 have morphological affinities with members of *Medicago*; again there is a parallel in phytoalexin synthesis in that *Medicago* species generally produce those compounds characteristic of the third *Trigonella* group. It is perhaps significant that taxonomists have made several attempts to move one member of group 3, namely *T. platycarpos*, into *Medicago*[9].

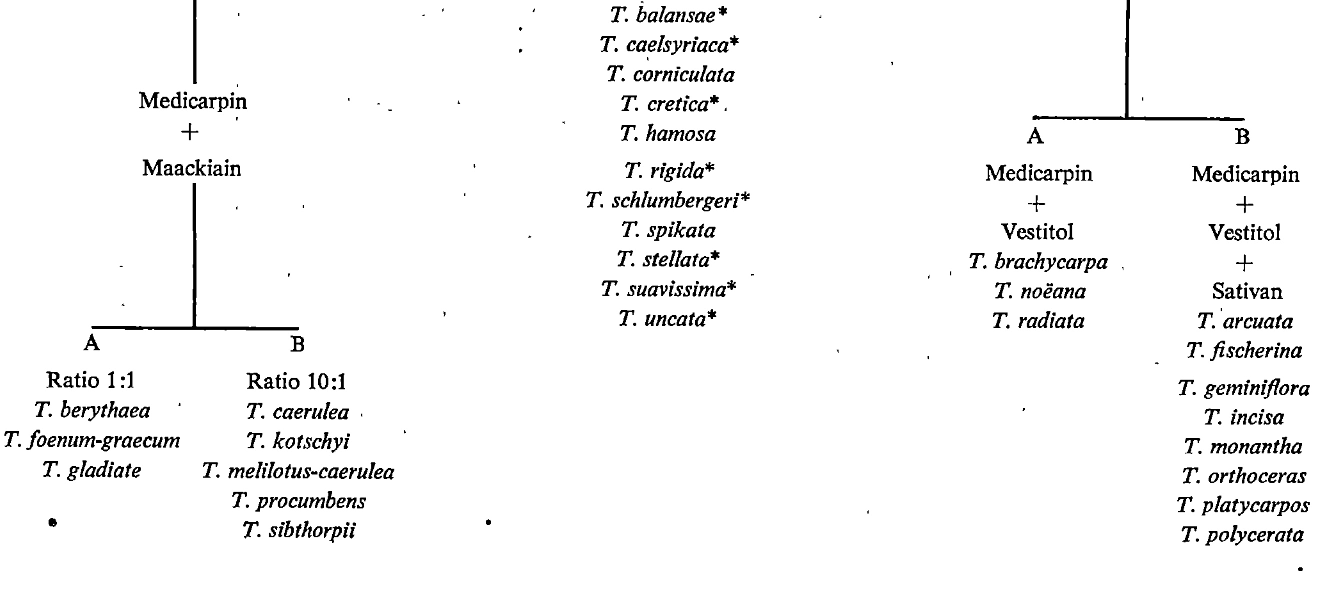


Fig. 1 Groupings of *Trigonella* species based on their phytoalexin production. *Coumarin producer.

The above results also have some bearing on evolutionary relationships between these taxa, in that several of the phytoalexins can be placed in a biogenetically developing series. Thus, medicarpin might be converted to vestitol, from which sativan could then be obtained by methylation. Production of maackiain is an evolutionary step of some significance as this chemically 'advanced' pterocarpan cannot be obtained directly from medicarpin[10]. At the same time is should be noted that both isoflavans (III and IV) are considerably more antifungal than are either of the pterocarpans. On this basis then, the three *Trigonella* groups fall into a series of increasing chemical advancement: group 2 → group 1 → group 3.

We report the present results because we believe that phytoalexin studies are generally applicable to systematic problems in angiosperms. We have successfully induced phytoalexin synthesis in twelve other leguminous genera and the results will be reported in detail elsewhere. Several known and new phytoalexins have been encountered in these genera, including resveratrol—a simple stilbene from groundnuts (*Arachis hypogaea*). Finally, we believe that phytoalexin surveys are economically important in that new naturally occuring compounds of potential value to crop protection may well be revealed.

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Received December 18, 1975; accepted January 12, 1976.

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Evidence of hormonal control of ovulation in tsetse flies

ODHIAMBO[1] and Saunders and Dodd[2] reported that the first fully developed egg in the tsetse fly (*Glossina pallidipes*[1] and *G. morsitans*[2]) is not released from the ovary into the uterus until the female has had mating experience. They observed that the component of the mating act causing ovulation was not insemination, and suggested that a mechanical factor is responsible for the initiation of ovulation[2]. It is difficult to see how a strictly nervous mechanism could account for the time lag between mating and ovulation (females mated 2–3 d after emergence usually ovulate 6–7 d later). Foster[3] found that the ablation of median neurosecretory cells (MNC) of the pars intercerebralis almost always inhibited ovulation in *G. austeni*. He noted, however, that surgical brain trauma also inhibited ovulation in control flies. Ejezie and Davey[4] found that the MNC undergo cyclic changes of net synthesis and release in *G. austeni* and attempted to correlate these phenomena with ovulation and larviposition. We describe here investigations of the control mechanism of ovulation in *G. morsitans morsitans*.

In our laboratory (24.5±0.5 °C, 65±5% relative humidity, 12 h light and 12 h dark and 100 lx at the level of experimental flies), females (fed on rabbit ears 6 d a week) are normally mated 2–3 d after emergence and ovulate 5–7 d later when they possess the first fully chorionated egg. But we found that if the females were prevented from mating until their first egg was fully chorionated (11–12 d after emergence, to be certain) and then were allowed to mate ('delayed' mated), they ovulated within 24 h of termination of copulation (Fig. 1).

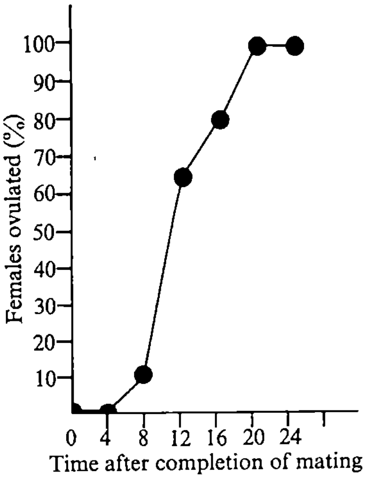

Fig. 1 Pattern of ovulation in delayed mated females. They were allowed to mate 12 d after emergence. Groups of 20 females were dissected 0, 4, 8, 12, 16, 20 and 24 h after termination of mating to determine whether ovulation had occurred.

A male could inseminate a female (while ovulated subsequently) when allowed to mate uninterrupted for a prolonged period—about 60 min. But brief copulatory acts of 20 min or less did not result in insemination or ovulation. To obtain conclusive data on the relationship of mating, insemination and spermatophore formation to ovulation, we allowed the female to experience multiple interrupted (manually separated) matings of shorter than normal duration (10–20 min) with several different males consecutively, which allowed her to accumulate a prolonged mating experience but prevented her from receiving sperm or sufficient male accessory reproductive gland (AG)

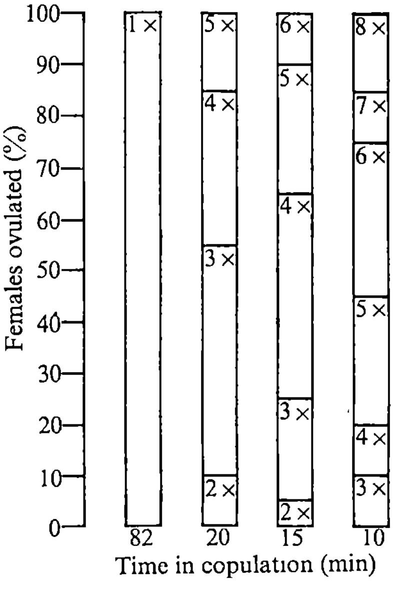

Fig. 2 Pattern of ovulation in females that experienced consecutive multiple matings of shorter than normal duration with fresh unmated males. Females (12 d old) were allowed to mate consecutively with two (2×) to eight (8×) males for 10, 15 or 20 min each by manually terminating each mating and allowing each female 30–100 min in copulation. Control females were mated with only one male (1×) and were not interrupted. Ovulation was checked 24 h after the last mating was terminated. Spermathecae were checked routinely to record any insemination. Each percentage is based on observations on 20 females.

secretion to form a spermatophore. Ovulation occurred in all females which spent 80–100 min copulating in several brief 'sterile' matings but the rate of ovulation declined with the decrease in copulation time (Fig. 2). Further observations of interrupted matings revealed that males could transfer some AG secretion during 10 min in copulation (unpublished work). To eliminate the effect of male AG secretion on ovulation, we removed the paired male AG, and, in some cases, the proximal part of the ejaculatory duct, and allowed the operated males to copulate with the virgin females. Twelve females which mated with the operated males for an average of 57 min ovulated, but five that mated for an average of 25 min did not.

There is no evidence of a male contact pheromone involved in induction of ovulation, as ovulation was demonstrated in virgin females which had no contact with males but into whose uteri one or two glass beads of about 300 μm diameter (about half the size of a spermatophore) had been inserted through the vaginal opening. A comparable effect is the sensory perception of artificial stimulation of the reproductive tract which results in ovulation in some vertebrates[5].

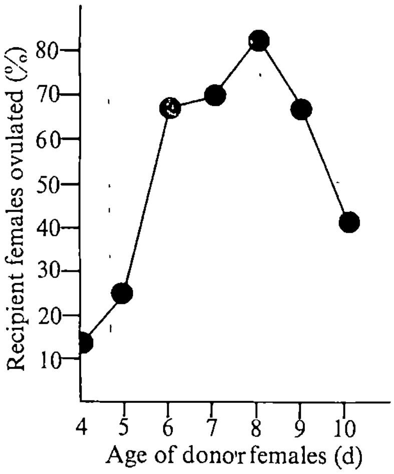

Fig. 3 Pattern of ovulation in virgin females after receiving haemolymph from the mated females of indicated age. Donor females were mated when 3 d old. Virgin recipients (n = 15–20) were 11–12 d old when they received haemolymph. Control virgin recipients (n = 10) received haemolymph from virgin donors. Ovulation was checked 48 h after the injection. Ovulation in controls = 0%.

We also removed the corpus allatum (CA) and corpus allatum–corpus cardiacum (CACC) of the female before or immediately after completion of mating. In either mating status the removal of CACC inhibited ovulation. Allatectomy of 9–12-d-old females did not interfere with the first ovulation.

Pursuing a different line of investigation, we induced ovulation in physiologically mature virgin females by injecting each of them with 0.5–0.8 μl haemolymph taken from 4–10-d-old mated (mated when 3 d old) females. Preliminary results show a correlation between the age of donor females at the time of transfusion and the percentage of recipients (n=15–20) showing ovulation (Fig. 3). Haemolymph from the virgin donors did not result in ovulation in virgin recipients (n=10).

We draw the following conclusions from these observations. (1) Ovulation is dependent on the physical component of copulation and (2) is independent of insemination and the transfer of male AG secretion or seminal fluid. (3) The duration of the mating act is critical in inducing ovulation. (4) The CC is important in the regulation of ovulation; (5) a blood-borne factor in the mated female is ultimately responsible for induction of ovulation, and (6) ovarian development itself may have a role in the control mechanism.

Our findings, together with earlier observations[1–4], suggest that afferent nervous impulses resulting from prolonged mating, and probably those resulting later from ovarian distension due to the presence of a fully developed oocyte, activate the MNC to release an ovulation-stimulating hormone into the haemolymph, probably through the CC. We suggest that this hormone ultimately induces ovulation.

We thank Professors Thomas R. Odhiambo, Vincent B. Wigglewsworth and Jan de Wilde for suggestions and Dr John Strangways-Dixon for reviewing the manuscript.

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Growth regulation in chimaeras between large and small mice

AGGREGATION chimaeras between strains of mice differing in body size may be used to determine whether the regulation of growth is determined by the relative proportions of cells deriving from the constituent strains. Should this be so, several questions arise; is growth regulated by the proportions in the body as a whole, or is the cellular composition of particular organs (or tissues) more critical? If critical organs or tissues are established, is the regulation then a systemic property, or does the response of other organs and tissues depend further on their own cellular composition? We describe here preliminary findings which indicate the answers to some of these questions and suggest ways of answering the others.

Aggregation chimaeras were made, by methods described previously[1], between mice from strains selected for large (L) and small (S) body size, and between L and unselected control (C) mice; the history of the strains is given in ref. 2. Some C$\longleftrightarrow$C chimaeras are included for comparison. Fifty overt chimaeras, all from C-strain foster mothers, were distributed among the three classes in the numbers shown in Table 1. Overt chimaerism was detected by marking the constituent strains with contrasting coat colours, either albino or coloured. Coat colours and body size were used reciprocally in chimaeras between L and S and since there was no difference between the reciprocals, they were pooled. In chimaeras between L and C, the latter was always the albino component. The proportion of albino in the coat was scored as described in ref. 3. We do not include data from successful aggregations which later resulted in single coat colours, but those mice ruled out any general tendency for L cells to outgrow S cells, or vice versa. This is supported by the fact that the mean proportion of L in the coat, in chimaeras both with S and with C, is close to 50% (Table 1). All data reported here were recorded when the mice were 6 weeks old. All female body weights were adjusted to male equivalents, males being 20% heavier in these strains. The weights of the constituent strains were adjusted further by regression to litter sizes equal to those in which the chimaeras were born, the weights thus seeming greater than those published earlier[2].

Examination of the data revealed a positive regression of body weight on the proportion of the L genotype in the variegation score of the coat (Fig. 1), the corresponding correlations being 0.51 and 0.70 in S$\longleftrightarrow$L and C$\longleftrightarrow$L chimaeras, respectively. Cumulatively, there is no doubt about the significance of these regressions, and two conclusions follow. First, body size is linearly and directly proportional to the cellular composition of the coat of the chimaera. Second, since melanocytes themselves can hardly be determinants of growth, the observed correlation must reflect a fundamental correlation between the proportions of melanocytes in the coat and the corresponding proportions of L and S cells in whatever tissue(s) regulate growth.

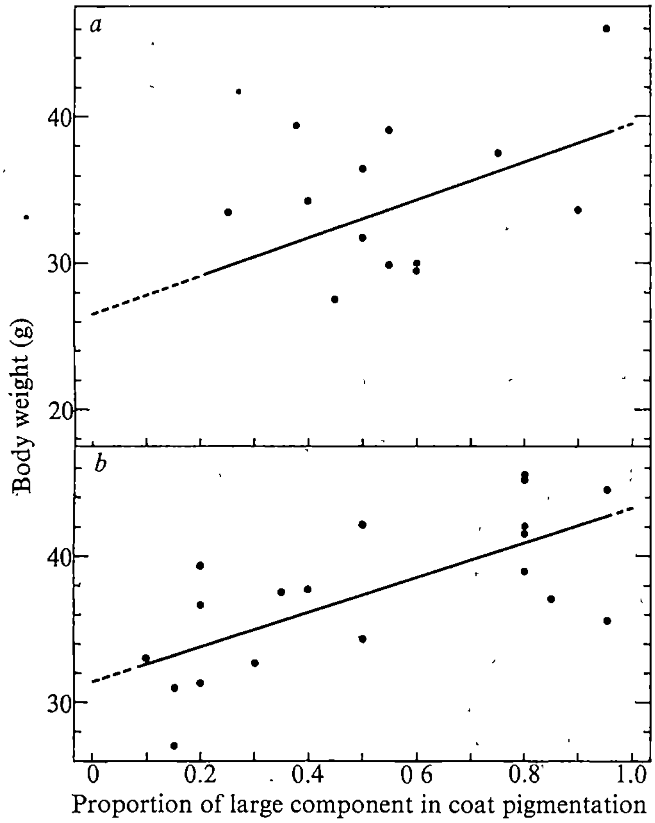

Fig. 1 Relationship between body weight and proportion of L component in coat pigmentation. Values of regression coefficients: in S⟷L (*a*), 13.0±6.9; in C⟷L (*b*), 11.7±2.9.

A critical observation from Table 1 is that S⟷L and C⟷L chimaeras are 2.5 times more variable in weight than C⟷C chimaeras, whose own variance is about the expected normal value. A source of variance in chimaeras between different strains, not present in other mice, is the variation between individuals in the proportions of the two cell types. For coat colour, this proportion varied between 0 and 1, and covered most of that range even if the single colours were excluded. If the same kind of variance in proportion applies to weight-controlling tissue, the inflated variance of weight is readily explicable. It is therefore important to estimate the variance of the proportions of cells in the weight-controlling tissue.

We now define the 'chimaeric genotype' (G) for growth in terms of the proportions of the two types of cells. Let

$$G = P\mathrm{L} + (1 - P)\mathrm{S}$$

where P is the proportion of large cells (L), leaving $(1 - P)$ small cells (S). In the other class of chimaeras, C is substituted for S. On rearrangement, this gives

$$G = \mathrm{S} + (\mathrm{L} - \mathrm{S})P = \mathrm{S} + DP$$

where D is the difference in weight between the constituent strains. G is thus a linear function of P, as implied by Fig. 1. Since S, for present purposes, is a constant,

$$\mathrm{var}(G) = D^2\,\mathrm{var}(P)\,,$$

where var(G) is the variance of G, and so on. Trivial reorganisation gives an expression for var(P)—the variance of proportions of cells in the growth-controlling tissue. D is directly observable and var(G) may be estimated, at least roughly, as follows. The variance in body weight of the C⟷C chimaeras does not contain any var(G), by definition, since G was defined in terms of the proportions of differing cell types; however, the variance of the C⟷C chimaeras will reflect all other sources of variation. Subtracting the variance in body weight of C⟷C chimaeras from that of the S⟷L chimaeras therefore provides a direct estimate of var(G) appropriate to that class. All the necessary data are available from Table 1, and using the expression derived above, we obtain estimates of 0.046 and 0.190 for var(P) in S⟷L and C⟷L chimaeras, respectively. Viewed slightly differently, these are the variances in the cellular composition of the growth-controlling tissue that would be necessary to explain the increased variance in weight found in chimaeras between different strains. Although we make no claims for the numerical accuracy of these estimates of var(P), the values found seem to be reasonable, and they correspond closely to the variance in the proportions of melanocytes, estimated from the coat by direct observation (see Table 1).

Table 1 Body weights and proportion of L component in coats

Constituent lines	L	C	S
Adjusted weights (g)	36.7	27.3	18.3
Chimaera type	S⟷L	C⟷L	C⟷C
n	12	19	19
Coat: proportion L	0.58	0.52	(0.28)*
variance	0.040	0.096	(0.075)*
Weight (g): mean	34.1	37.5	28.2
variance	25.7	26.8	10.0

* These values refer to the proportion of albino in coat.

Our results therefore establish that growth in chimaeras is a direct function of the proportions of cells from the two constituent strains, which in turn implies that growth regulation resides in the cellular properties of certain organs or tissues. Further, from consideration of the variances of the proportions of cells, it seems that the system of growth regulation needs be neither more complicated nor more extensive than that controlling the distribution of melanocytes in the skin. This indicates that the organs or tissues governing growth control may eventually be located, and to that aim we are marking constituent strains with variants of an enzyme that can be assayed quantitatively. The higher the correlation between body weight and proportion of L cells in various tissues, the more important will that tissue be in regulating growth. Equally, the systemic effect deriving from such tissue can be examined against the cellular composition of target organs, to see whether there is any local autonomy of size regulation

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Received October 17, 1975; accepted February 9, 1976.

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Evidence for a new diffusible element of mating pheromones in yeast

THE yeast *Saccharomyces cerevisiae* produces pheromones which seem to participate in the mating process[1,2]. Each of the two haploid mating types (*a* or α) produces a diffusible factor (called *a* factor or α factor, respectively) which causes cells of the opposite mating type to be retarded in the G_1 period of the cell division cycle[3–6]. The pheromones thus act to synchronise the cell cycles of mating cells before the formation of the diploid zygote. By a variation of the original assay for yeast mating pheromones[8,9], we have observed that cells of mating type *a* produce a new pheromone which inhibits action of the α factor.

The standard assay for the α factor requires the placing isolated *a* cells ('assay cells') near a source of α factor, for example, a heavy streak of α cells[8,9]. The α cells are unable to initiate new rounds of budding[2] and instead undergo the α-factor response, a characteristic shape change (see Fig. 1*c*, *d* and *e*). Continued exposure to α factor results in the further elongation of the *a* cell for at least 12 h, at which time cells furthest from the source of the α factor resume budding (ref. 4, and R. Chan, L. Hartwell and T. Manney, personal communication).

When a dense streak of *a* cells is placed between the α cells and *a*-assay cells (Fig. 2*a*), no α-factor response is observed in the *a*-assay cells (although the response is seen within the streak of *a* cells). Figure 1 shows the response of *a*-assay cells with (Fig. 1*a*) and without (Fig. 1*e*) interposed cells. The interposed cells therefore exhibit a barrier to the α-factor response and are said to have the Bar$^+$ phenotype. To determine whether the barrier effect is controlled by the mating type locus, we have examined the Bar phenotype of a number of different *a* strains, *a*/α strains, and two classes of α mutants (*ste*$^-$) which do not produce the α factor (ref. 7 and J.M.H. and I.H., unpublished). All of the *a* strains tested here are Bar$^+$, and all the *a*/α strains are Bar$^-$. Although the barrier phenotypes of the *a* strains and the *a*/α strains are clearly different, it has been observed that assay cells in the presence of an *a*/α barrier recover from α-factor inhibition more quickly than controls where no barrier is present. This may indicate the partial expression of the Bar function in *a*/α diploids. (Analogous observations have been made by T. R. Manney (personal communication).) Interestingly, the α strains carrying *ste*$^-$ mutations inseparable from the mating type locus (classes 1 and 2) differ in their Bar phenotype. It is thus clear that the mating type locus itself regulates the barrier effect.

To determine further the relationship between the barrier effect and genes involved in mating, we examined the Bar phenotype of *a* cells carrying mutations which block mating (*ste*$^-$). Six of the *a ste*$^-$ are Bar$^+$, and three are Bar$^-$ (see Fig. 1*b* and *c*). The barrier effect therefore seems to be under the control of genes essential to mating. Since all of the *a ste*$^-$ mutants tested were insensitive to the α factor (ref. 7, and unpublished), there is no correlation between the Bar phenotype and α-factor response. The sterile mutant of class 10 behaves in some respects, including its Bar phenotype, as an *a* mutant (see Table 1). It should be noted that the existence of Bar$^-$ strains (*a*/α, α *ste*$^-$, and some *a ste*$^-$) argues against the notion that the barrier effect is attributable to a nonspecific effect of the growth of cells in the barrier streak (Table 1).

The barrier effect could involve the adsorption of the α factor by the *a* cells within the barrier streak. Another possibility (not exclusive of the first) is that the barrier cells produce a 'diffusible barrier', that is, a diffusible inhibitor. To test that possibility, we rearranged the geometry of the standard α-factor assay so that the *a*-assay cells were placed between heavy streaks of α and *a* cells (the 'N technique', see Fig. 2*b*) Under such an arrangement, the assay cells furthest from the *a* streak responded to α factor, whereas those closest to the *a* streak did not. The presence of the *a* streak was not essential for the inhibition of the α-factor response. Rather, *a* cells seem to excrete an inhibitor into the agar. This diffusible effect can be seen by allowing a streak of *a* cells to grow on an agar slab for 6 h at 30 °C. The *a* cells should then be removed by cutting the agar (see Fig. 2*b*). The standard α-factor assay should then be carried out on the slab thus obtained using *a*-assay cells at varying distances from the α cells. As controls, the assay should be carried out in parallel on untreated agar, and on agar 'treated' by growth of an *a ste*$^-$ strain which is Bar$^-$ (strain S111). As before, the *a* cells nearest to the α cells will respond to the α factor in all

Table 1 Ability of different strains to exhibit barrier and diffusible effects on response to α factor.

Strain*	Mating type	Barrier effect	Diffusible effect	*a* factor†
39, 73, 75, 94, X5–39	*a*	+	+	NT
S107, S111	*a ste*$^-$	–	–	NT
S110	*a ste*$^-$	+	–	NT
S108	*a ste*$^-$	+	NT	NT
VAB2 (class 3), VW9 (class 7)	*a ste*$^-$	+	+	+
VAB1 (class 5), VU3 (class 15)	*a ste*$^-$	+	+	–
VY3 (class 13)	*a ste*$^-$	–	–	+
VC73 (class 10)	( )‡	+	+	NT
VC2 (class 1)	α *ste*$^-$	–	–	NT§
VB3 (class 1)	α *ste*$^-$	–	NT	NT§
VP1 (class 1)	α *ste*$^-$	+	NT	NT§
VN33 (class 2)	α *ste*$^-$	–	–	NT§
X14, X16, X17	*a*/α *ho*/*ho*	–	–	NT
X47	*a*/α *HO*/*ho*	–	–	NT
X10–1B	*a*/α *HO*/*HO*	–	–	NT

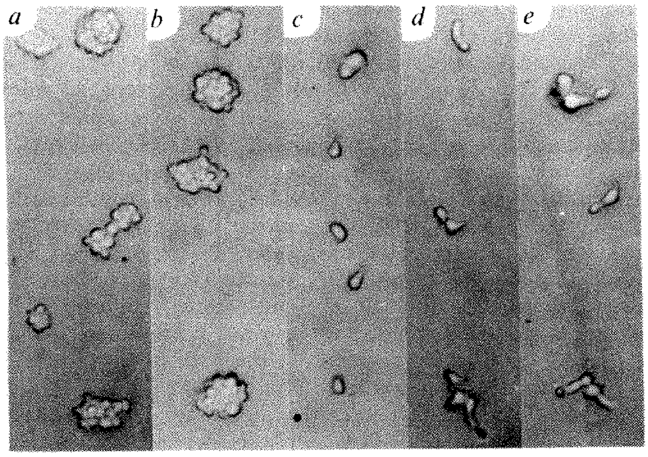
In most cases the *a*-assay cells were strain 73 (*a-ilv*3–X) and the α-factor source cells were strain 70 (α *thr*3–10).

*Strains 73, 75, 94, X5–39 and X10–1B are described in ref. 10. S107, S108, S110, and S111 are sterile mutants isolated from X5–39, and carry mutations *ste*107, 108, 110, and 111, respectively (unpublished). The V series sterile mutants are described in ref. 7.

†Ref. 7.

‡VC73 was isolated from an α parent and does not produce the α factor[7]. It mates with α cells 1% as efficiently as do wild-type *a* cells (unpublished).

§These mutants do not produce the α factor.



75 (a^+) S 110 (a ste^-, Bar$^+$) S 107 (a ste^-, Bar$^-$) X-14 (a/a) Control (No barrier)

Fig. 1 The α-factor response by *a*-assay cells (strain 73) in five separate barrier tests photographed after 12 h incubation at 30 °C. The strain designations below each panel refer to the barrier cells. Arrangement as in Fig. 2*a*. Growth of assay cells in (*a*) and (*b*) is the same as in the absence of α cells (data not shown) and indicates the absence of α-factor activity. The production of morphologically aberrant cells in *c*, *d*, and *e* is the normal response to the α factor exhibited by *a* cells. The α-factor source was strain 70.

cases. The α cells further from the α streak, however, will not exhibit the α-factor response on agar treated previously with *a* cells. On untreated agar or on agar treated by the growth of the *a* ste^- strain, all of the *a*-assay cells will exhibit the α-factor response. This indicates that *a* cells produce a diffusible inhibitor of the α-factor response.

To test whether the inhibitor acts on α cells (for example, on α-factor synthesis or release), we looked for inhibition in the absence α cells when the α factor was present in the agar. The α cells were grown on an agar slab (Fig. 2*b*) for 6 h, and were then removed by cutting away an agar slab. The slab was cut in half horizontally (Fig. 2*b*) to give slabs A and B. A dense streak of *a* cells was laid down on slab A, and a streak of *a* ste^- Bar$^-$ (S111) was laid down on slab B. The α-factor response by *a*-assay cells placed on slab A was considerably less than the response on slab B. The diffusible inhibitor can, therefore, act in the absence of α cells.

The relationship between the Bar$^+$ effect and the diffusible inhibitor was examined by testing a number of strains for the diffusible inhibitor by the N technique (Fig. 2*b* and Table 1). No Bar$^-$ strains (a/α, *a* ste^-, or α ste^-) exhibited a diffusible effect. All the strains tested which showed a diffusible effect were Bar$^+$ but not vice versa. We observed one case in which a Bar$^+$ strain (S110) did not show the diffusible effect. Further analysis of this mutant and characterisation of more *a* ste^- mutants will be necessary in order to determine the relationship between the barrier effect and the diffusible inhibitor.

Therefore, *a* cells are able to block in some manner the action of the α factor. The ability of strains to exhibit a barrier effect is controlled by the mating type locus and by genes essential for mating, suggesting that it may be a normal component of the mating process. The observation that class 1 sterile mutants differ in their Bar phenotypes provides further evidence for the complexity of the mating type locus. At least part of the barrier effect is mediated by a diffusible factor. This factor does not necessarily act on the synthesis or release of the α factor by α cells since its action is seen in the presence of the α factor. The inhibitor, thus, may act directly on *a* cells to modify their response to the α factor itself. The production of the inhibitor does not require induction by the α factor. Finally, the inhibitor seems to be distinct from the *a* factor since some strains which are *a*-factor deficient produce the inhibitor and vice versa (Table 1).

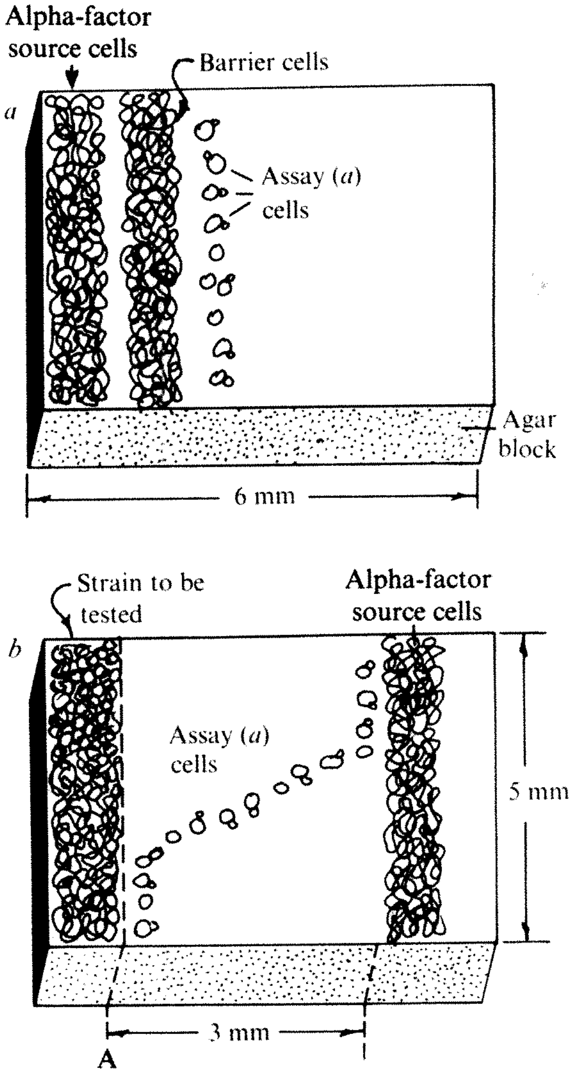


Fig. 2 Experimental arrangement for studying the barrier effect (*a*) and the diffusible inhibitor (*b*) of response to the α factor. Cells were pregrown overnight at 30 °C on YEPD agar (2% peptone, 1% yeast extract, 2% glucose, 2% agar). In the barrier test, α cells ('α-factor source') and barrier cells were applied to blocks of dissection agar (2% peptone, 1% yeast extract, 4% glucose, 4% agar) in streaks containing approximately 10^7 cells. The blocks were placed on a glass coverslip and inverted over a dissection chamber. From 10 to 20 individual mating type *a* cells ('assay cells') were then placed by micromanipulation on the agar in the position shown, 2±0.2 mm from the source cells, and the chamber was sealed to prevent any loss of moisture. The growth rate and morphological characteristics of the cells were used as an assay of α-factor activity in the agar. The presence of α-factor activity in the agar distal to the barrier cells was indicated by distorted cell morphology (shmooing) and the absence of cell division by the assay cells after 12 h incubation at 30 °C (Bar phenotype; Fig. 1: *c*, *d*, *e*). The absence of α-factor activity was indicated by the ability of the assay cells to divide normally and form microcolonies (Bar$^+$ phenotype; Fig. 1: *a* and *b*). To determine whether strains produce a diffusible inhibitor of α-factor response, the streak of cells to be tested was applied to each agar block first as in arrangement *b*. After growth for 6 h at 30 °C, the section of agar containing this streak was cut away (along dotted line A in the diagram), and α-factor source cells and *a*-assay cells applied to the block as shown. The response of the assay cells was observed microscopically at 6, 12, and 24 h.

What would be the role of the barrier effect and diffusible α-factor inhibitor in the normal mating process? We offer three suggestions. First, the inhibition of α-factor response may be part of the mechanism by which *a* cells recover from an α-factor block[4] and by which newly-formed a/α zygotes escape from inhibition by *a* and α factors in their environment. Second, successful mating between individual cells requires cell contact. The α factor can, however, act at a distance to inhibit the growth of *a* cells. The 'anti-α factor' activity may reduce the effective concentration of the α factor in the immediate environment of an *a* cell so that only the *a* cells very close to α cells will respond.

Third, the successful mating between populations of haploid cells requires equal numbers of cells of each mating type. In situations in which the number of *a* cells is greater than the number of α cells, mating would result in a small number of diploid cells and a large number of unmated *a* cells. If, however, the response to the α factor is blocked by a high concentration of the inhibitor, mating could be delayed until the further growth of α cells has occurred. The number of *a* and α cells would thus become comparable, and a greater fraction of the population could mate successfully.

We thank Jeff Strathern and Rick Dahlquist for discussions and suggestions, and Tom Manney, Russell Chan, and Lee Hartwell for strains, unpublished information and discussion. This work has been supported by an NIH Program Project Grant to the Institute of Molecular Biology and by an NIH Molecular Biology Training Grant (J.B.H.).

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Received August 28, 1975; accepted January 19, 1976.

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Regulation of the fibroblast cell cycle by serum

WHEN "normal" fibroblasts are deprived of serum they cease growing and accumulate in the G_1 or G_0 stage of the cell cycle[1]. On restoration of serum, the cells begin to enter S phase quasi-synchronously after a well defined lag period which seems to be characteristic of the cell type. Following the studies of Todaro *et al.*[2], Bürk[3], and Temin[4], it is widely believed that exposure to serum for only a portion of this lag period is sufficient to commit the cells irreversibly to initiate a round of DNA replication. Thus, removal of the serum after only a few hours, and before the end of the lag period, does not affect subsequent DNA synthesis in a significant proportion of the population. It has been apparent, nevertheless, that the proportion of such "committed" cells depends on the duration of the serum pulse[2–5]. Indeed, the relationship between the length of exposure to serum and the proportion of committed cells is approximately linear with time and (with 3T3 cells) extends beyond the end of the lag phase itself (Fig. 1). Clearly, some cells which are stimulated to initiate DNA synthesis during continuous exposure to serum are not committed by the end of the lag period, since removal of the serum at this point reduced the proportion of labelled cells, at 24 h, from 76 to 53% (Fig. 1*b*).

Experiments such as these have been interpreted as evidence that some cells require longer periods of mitogenic stimulation than others to become irreversibly committed to division[6]—a view which is apparently reinforced by the well known asynchrony with which cells begin DNA synthesis, even when the mitogen is present continuously. Recently, however, it has been shown[7] that this asynchrony is not necessarily evidence of cellular heterogeneity but may be a consequence of the very nature of the commitment event itself[7]. In the presence of micromolar concentrations of metabolisable purines or purine nucleosides (which potentiate the serum response[8,9]) the rate of entry into S phase increased very abruptly after the lag period, rapidly becoming first order[7]. Thereafter, a constant fraction of the G_1 population began DNA synthesis per unit time. This suggested that commitment was a random event occurring with a characteristic probability, as proposed earlier by Smith and Martin on other grounds[10]. The rate constant (or transition probability) for entry into S phase was found to be a function of the serum concentration, which means that this determines the rate of commitment rather than the number of committed cells. In the absence of exogenous purines, the rate constant increased slowly with time, but in their presence, the new value was attained abruptly with

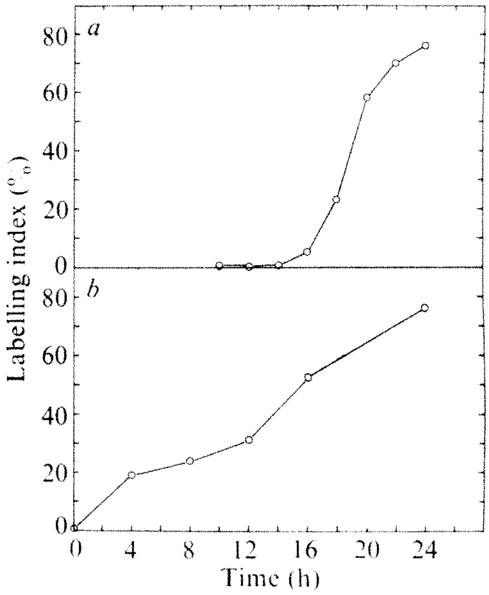

Fig. 1 *a*, Time course of entry into S phase after stimulation of quiescent 3T3 cells with 4% serum. Subconfluent, quiescent cultures of (Swiss) 3T3 cells[13] were prepared by plating 3×10^4 cells on to 15-mm glass coverslips in each well of a Linbro multidish tray (Linbro 24-16-TC), in 1 ml of 0.5% calf serum in Vogt and Dulbecco's modified Eagle's medium[14], followed by incubation at 37 °C for 3 d. Coverslips were then transferred to 3-cm plastic Petri dishes containing 2 ml of fresh medium and 4% calf serum (previously dialysed against 2×50 volumes of isotonic phosphate-buffered saline), together with inosine (25 µM) and ^{3}H-thymidine (1 µCi ml^{-1}; 3 µM thymidine). With this ratio of medium volume to cell number, depletion or inactivation of serum activity[15,16] is not significant during the subsequent course of the experiment.) At the times indicated, pairs of coverslips were removed and processed for autoradiography as described previously[7] using Kodak NTB3 nuclear track emulsion. After exposure for 2 weeks at 4 °C, autoradiographs were developed, stained with Geimsa solution and the proportion of cells with labelled nuclei determined. *b*, The effect of serum pulse length on the proportion of cells in S phase at 24 h. Quiescent 3T3 cells were stimulated with 4% dialysed calf serum in medium containing inosine (25 µM) and ^{3}H-thymidine (1 µCi ml^{-1}; 3 µM thymidine). At the times indicated, pairs of coverslips were transferred to fresh medium containing 0.25% dialysed serum, but with the same concentrations of inosine and ^{3}H-thymidine. After 24 h all coverslips were removed and prepared for autoradiography. The abscissa represents the duration of exposure to 4% serum.

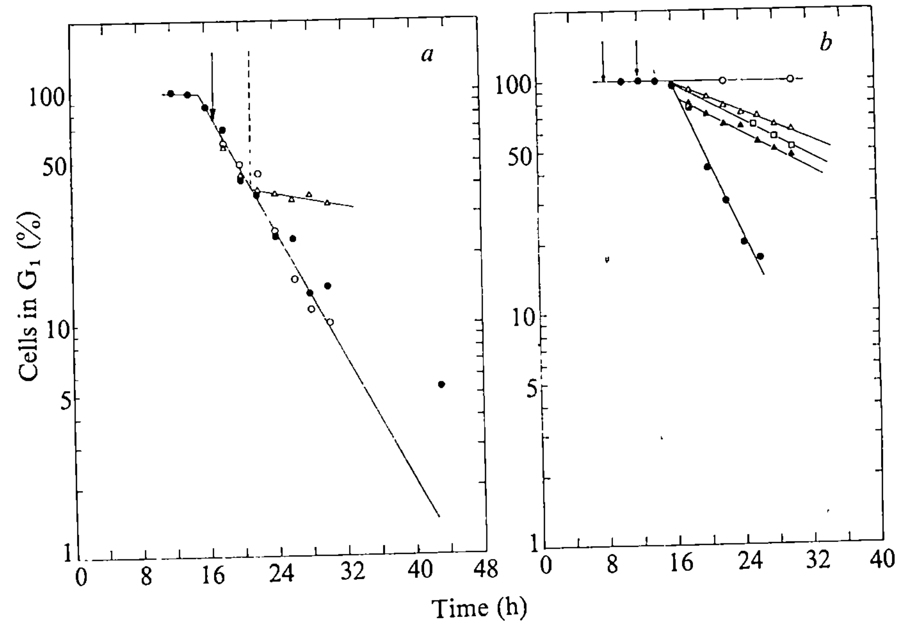

Fig. 2 *a*, The effect of serum step-down on the rate of entry into S phase. Quiescent 3T3 cells were stimulated with 10% dialysed calf serum in the presence of inosine (25 μM) and ^{3}H-thymidine (1 μCi ml^{-1}; 3 μM thymidine). After 16 h, some coverslips were washed with warm (37 °C) serum-free Dulbecco's modified Eagle's medium and transferred to fresh medium containing the same concentration of inosine and ^{3}H-thymidine and either 10% dialysed serum (○) or 0.25% dialysed serum (△). Other coverslips remained untouched (●). Pairs of coverslips from each set were removed at the times indicated and processed by autoradiography. The labelling index was determined and the proportion of cells remaining in G_1 obtained by subtraction, except for the point at 44 h which was calculated by counting the number of unlabelled cells per field and estimating the original cell density (that is, before cell divisions) from the preceding coverslips. *b*, As for (*a*) except that the highest serum concentration used was 4%, and the step-down to 0.25% made at either 8 h (△) or 12 h (▲). ○, 0.25%; □, 1%; ●, 4%. For clarity, the early points for the 1% serum control (□) have been omitted.

little evidence of passing through intermediate values. Once attained, the new value remained constant for at least a further 30 h (unpublished data). This suggested that the probability of initiating the cell cycle might reflect an equilibrium steady state in the rate of some cellular activity which in turn was controlled by the serum concentration. According to this interpretation, maintenance of the transition probability is regarded as a dynamic process dependent on the continuous presence of the growth stimulus, while much (and perhaps all) of the lag period is considered to be the time required to alter this equilibrium state. This hypothesis predicts that a reduction of the serum concentration at any point will be accompanied, some time later, by a decline in the rate of initiation.

Evidence in favour of this interpretation has been obtained by measurement of the proportion of cells remaining in G_1 at various times following stimulation. When the serum concentration was reduced from 10 to 0.25% at 16 h, the rate of initiation declined abruptly 5 h later (Fig 2*a*). This decline was not the nonspecific result of manipulations involved in changing the medium but depended on the alteration of serum concentration. In other experiments, serum step-downs have been made after 12 h (Fig. 2*b*) or 20 h exposure (not shown): in each case the rate of initiation declined to a new steady state between 3 and 5 h later, regardless of how many cells had reached S phase at the time of the perturbation.

Following the step-down, the rate of entry into S phase does not decline to the very low value of the control (0.25% serum continuously), but remains elevated at a rate approximately equivalent to 1% serum. Whether this is the result of serum growth factors remaining absorbed to the cell surface in a manner that survives washing, or to some other reason, is not known This residual rate of initiation shows some dependence, however, on the duration of the preceding serum pulse. When the step-down is made more than 5 h before the end of the lag phase, it is precisely this residual rate of initiation which seems to be responsible for the apparent "commitment" described by others following short serum pulses[2-5] (Fig 2*b*). As when serum is present continuously, the number of cells stimulated to begin DNA synthesis depends on the interval of time considered.

These experiments indicate that the apparent simplicity of the relationship between the length of exposure to serum and the proportion of committed cells (Fig. 1*b*) conceals a complex situation involving both the interval of observation and changes in the rate of initiation. There is no compelling evidence for subpopulations of hypersensitive cells poised, to varying degrees, close to the commitment step and requiring only short serum stimulation. The experiments also suggest that commitment to DNA synthesis in 3T3 cells does not occur earlier than 5 h before S phase, regardless of when initiation actually takes place in each cell relative to serum addition. This must be so because otherwise it would not be possible to prevent the initiation of DNA synthesis in a committed cell by withdrawal of serum unless there were some additional serum-dependent step between commitment and the start of S phase. This possibility can be dismissed because the initial lag period between serum addition and the beginning of DNA synthesis—which, by definition must include such a hypothetical step—is completely independent of serum concentration[4,7].

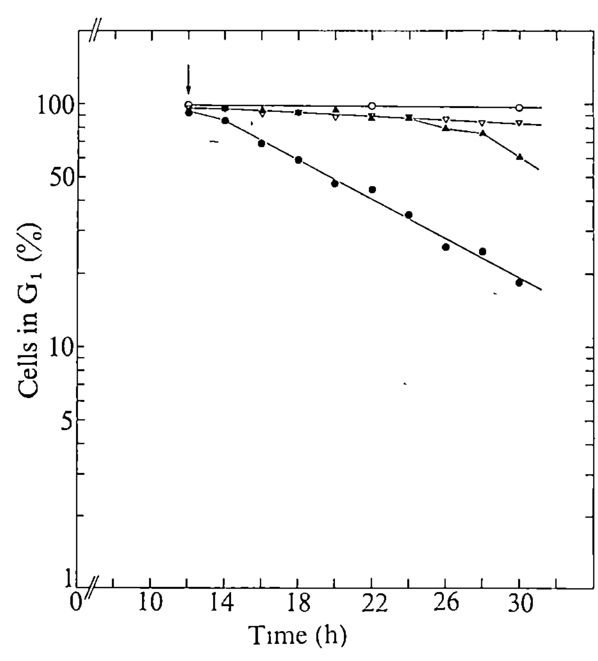

Fig. 3 The effect of serum step-up on the rate of entry into S phase. Quiescent 3T3 cells were stimulated with either 1 (▽) or 4% (●) dialysed serum. After 12 h some of those coverslips which had been placed in 1% serum were transferred without washing to fresh medium containing 4% dialysed serum (▲). (○, 0.25% serum). At the times indicated, pairs of coverslips from each set were removed and processed by autoradiography. The labelling index was determined and the proportion of cells remaining in G_1 obtained by subtraction Inosine (25 μM) and ^{3}H-thymidine (1 μCi ml^{-1}; 3 μM thymidine) were present throughout.

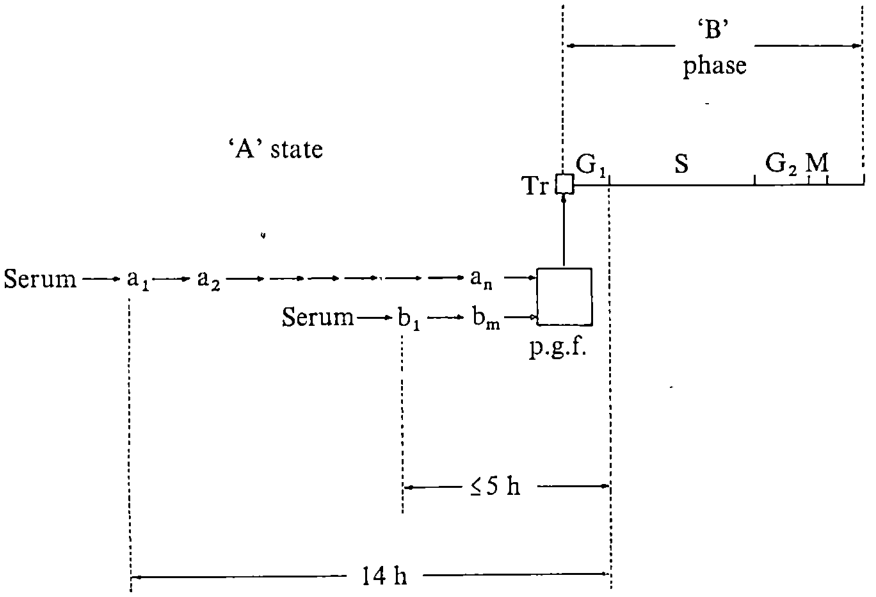

Fig. 4 A schematic view of the regulation of the cell cycle by serum. Serum concentration is considered to control the rate of at least two independent, convergent sequences of interconnecting, on-going processes (a or b). The point of convergence has been drawn, arbitrarily, at an hypothetical "probability generating function" (p.g.f.) postulated to activate a "trigger" (Tr) which, in turn, establishes the commitment to divide. Following commitment, the series of deterministic events leading up to mitosis have been labelled 'B' phase, after Smith and Martin[10]. All other processes are considered to take place in G_0 or 'A' state[10], though these activities do not necessarily cease after commitment has occurred.

Although the commitment event cannot occur more than 5 h before S phase, however, the present data are not incompatible with an even closer coincidence with the beginning of S phase, as suggested recently by Rubin and Steiner[11], and by Smith and Martin[12].

In contrast to the effect of a serum step-down, which is manifest within 5 h, when the serum concentration is raised from a subsaturating level (1%) to a higher serum concentration (4%), no further increase in the rate of initiation is observed until after another 12–14-h lag (Fig. 3). This is the case whether the step-up is made after 12 h (Fig. 3) or 8 h, or from a higher initial serum concentration (not shown), and is thus independent of previous stimulation. Step-ups have not been made later than 12 h because continuation of such experiments beyond 30 h is complicated by the appearance of mitoses which render the cumulative labelling index less meaningful. For this reason, it is therefore not possible, at present, to prove rigorously that the final rate of initiation after step-up is identical to that produced by the higher serum concentration when present from the beginning. From the available data, however, this seems to be a reasonable supposition.

It is thus evident that the 14-h lag represents the time required to effect any increase in the probability of commitment, regardless of the initial value. Nevertheless, the transition probability decreases within 5 h on withdrawal of serum. This disparity suggests that serum controls at least two distinct cellular activities necessary for the maintenance of the transition probability. This is depicted schematically in Fig. 4 as two independent sequences of interconnecting processes, arbitrarily drawn to converge at the "probability generating function" (p.g.f.). Serum is considered to regulate the rate of the initial process in each sequence (a_1 or b_1) while the lag after either step-up or step-down is determined by the relaxation time for the respective sequences (a or b) as a whole to reach the new steady state. In the sense that each constituent process is viewed as ongoing (with serum concentration determining the rate), they should not be considered as events through which the cell must progress. Indeed, they should be regarded as outside of the cell cycle in G_0 (the 'A' state of Smith and Martin[10]), in so far as they occur before the commitment step. The value of the transition probability would seem to be set by the longest sequence, while the shorter is presumably concerned with some permissive function. It will be interesting to determine whether the processes a_1 and b_1 are regulated by different serum factors. The possible basis of the p.g.f. has been discussed previously[12]; further speculation is inappropriate here.

I thank Dr R. W. Holley for facilities and discussion. This work was supported in part by grants from the National Science Foundation, National Institutes of Health and the American Cancer Society. I am a Fellow of the Leukemia Society of America, Inc.

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Received October 14, 1975; accepted January 9, 1976

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T-lymphocyte response to Friend virus-induced tumour cell lines in mice of strains congenic at *H–2*

Mice immunised with infectious lymphocytic choriomeningitis virus (LCMV) produce T lymphocytes which can kill LCMV-infected target cells *in vitro* only if the killer and target cells are syngeneic at the *H-2K* and/or *H-2D* loci[1,2]. We now report analogous results obtained in studies of the immune response of syngeneic or semi-syngeneic mice to cultured cell lines derived from tumours induced *in vivo* by Friend murine erythroleukaemia virus (FV) Our studies further indicate that the *H-2* type of the cells used for immunisation is also a decisive factor in the specificity of the killer cells generated.

The mice used were of strains congenic at the *H-2* region on the BALB/c (*H-2*^{d}) background: BALB.B

Table 1 T-killer cells in PEC from mice immunised with syngeneic or semi-syngeneic FV-induced tumour cells

Mice immunised*	Tumour cells used for immunisation and target cells	% ^{51}Cr release following various pretreatments of the PEC† *A*	*B*	*C*	*D*
BALB.B	HFL/b	30	1	36	30
BALB.K	HFL/k	10	2		
(BALB.B × BALB.K)F$_1$	HFL/b	20	4		
(BALB.B × BALB.K)F$_1$	HFL/k	45	8		

In the LMC, 2.5×10^6 PEC in 1 ml and 5×10^4 ^{51}Cr-labelled tumour cells in 0.1 ml (PEC–target cell ratio, 50 : 1) were mixed and incubated at 37 °C in 5% CO$_2$ atmosphere for 6 h. The cells were suspended in Medium 199 plus 20% foetal calf serum. Specific release of radioactivity into supernatant was compared with that from tumour cells alone frozen and thawed four times.

*PEC were collected at previously determined peaks of activity, 5 d after the second or third immunisation except for PEC obtained from the BALB.K anti-HFL/k immunisation which were collected after 7 d. Immunisation was done intraperitoneally with 5×10^6 tumour cells suspended in normal saline except in the case of BALB.K anti-HFL/k for which all immunisations after the first were with 10^7 cells.

†Pretreatments: *A*, normal mouse serum plus complement; *B*, mouse anti-Thy-1 2 plus complement; *C*, goat anti-mouse IgG plus complement; *D*, absorption on washed nylon column 10^7 PEC were first incubated at 4 °C for 30 min in 0.4 ml of antiserum (1 : 2 dilution), then resuspended and incubated at 37 °C for 40 min in 0.6 ml of guinea pig serum (1 : 3 dilution) as a complement source.

(*H-2^b* from C57BL/10), BALB.K (*H-2^k* from C3Hf/An) and the F$_1$ cross from these two strains (BALB.B × BALB.K) F$_1$ The cultured tumour cell lines induced in mice of these strains were of the HFL series: HFL/b (BALB B), HFL/k (BALB.K) and HFL/bk [(BALB.B × BALB K)F$_1$] (ref. 3). All three cell lines express both the FV-induced FMR cell-surface antigen and the virus envelope antigens associated with the gp70 viral protein[4]. Each of the cell lines readily induces transplantation immunity in syngeneic hosts, with concomitant immunity to spleen focus induction by FV.

BALB B, BALB.K and F$_1$ mice were immunised by intraperitoneal inoculation with *H-2*-compatible cultured tumour cells. Peritoneal exudate cells (PEC) from these mice, taken on day 5 or 7 after the second or third immunisation, were assayed for lymphocyte-mediated cytotoxicity (LMC) by the method of Berke *et al.*[5]. Targets for this assay were ^{51}Cr-labelled cultured tumour cells or phytohaemagglutinin (PHA)-induced blasts originating from mice of the same congenic strains. The blasts were obtained from spleen cells incubated at 37 °C with PHA (1 : 100) for 3 d in an atmosphere of 5% CO$_2$.

Tables 1 and 2 show the results of our studies. LMC was completely abolished by pretreating the immune PEC with anti-Thy-1 2 and complement, whereas pretreating the immune PEC with normal mouse serum or goat anti-mouse IgG and complement or incubating the PEC at 37 °C for 45 min on a washed nylon column had no effect on ^{51}Cr release (Table 1). Thus, target-cell killing in this system was mediated by T lymphocytes.

In agreement with other studies[1,2,6], we observed a requirement for shared *H-2*-region genes between the killer and target cells PEC from BALB B mice immunised with HFL/b tumour cells killed HFL/b and HFL/bk but not HFL/k target cells, and PEC from BALB.K mice immunised with HFL/k tumour cells killed HFL/k and HFL/bk but not HFL/b target cells. PHA blasts of either *H-2^b* or *H-2^k* type were not killed.

In previous studies of cellular immunity to virus-infected cells, the animals have been immunised directly with infectious virus, which is, in effect, an immunisation with autochthonous infected cells[1,2,6]. Since our immunisations were performed with cultured virus-induced tumour cells, we could study the response of F$_1$ mice immunised with parental strain tumour cells. From these experiments (Table 2) we have observed that T-cell killing also requires at least partial *H-2* region identity on the part of the immunising cells and the tumour cells used as targets. Thus, PEC from (BALB.B × BALB.K)F$_1$ mice immunised with HFL/k killed only HFL/k and HFL/bk but not HFL/b target cells, although the immune PEC possessed an *H-2* haplotype in common with all three target cells. Similarly, PEC from F$_1$ mice immunised with HFL/b killed HFL/b but not HFL/k target cells. These findings are analogous to those of Shearer *et al.* in which the T-killer cell response of F$_1$ mice to TNP-modified parental strain spleen cells was studied[7].

A comparison of the responses of BALB.K and of F$_1$ mice to HFL/k cells suggests the involvement of an *H-2*-associated immune response gene. In BALB.K mice the peak response was reached on day 7 rather than day 5, required a larger immunising dose of tumour cells and produced a lower level of ^{51}Cr release in tests on HFL/k target cells (Tables 1 and 2). It is conceivable that the presence of the *H-2^b* haplotype in the recipient confers a greater capacity for this immune response than the *H-2^k* haplotype.

To explain the requirement for common *H-2* genes between T killer and target cells in virus-specific systems, two mechanisms have been proposed: (1) compatibility at *H-2* is a physiological requirement for T-killer cell–tumour cell interactions specific for viral antigens; or (2) an alteration of the *H-2* molecules on cell surfaces occurs as a result of virus infection, and these altered molecules, rather than viral antigens *per se*, are recognised as "non-self" by responding T-cell populations.

The first of these possible mechanisms implies that killer cells from (BALB.B × BALB.K)F$_1$ mice immunised with either HFL/k or HFL/b cells should kill both HFL/k and HFL/b cells since these killer cells share *H-2* determinants with both parental strain tumour cell lines. Since F$_1$ T lymphocytes killed only the parental tumour cell with which they were immunised, this hypothesis does not seem correct.

If, according to the second proposed mechanism, *H-2* molecules are altered as a result of the cell's infection by FV, two possible types of alteration may be envisaged: either the primary structure of the *H-2* molecule itself is altered (pre-translational modification) or an interaction takes place between a viral molecule and an *H-2* molecule (post-translational modification). In either case, the modification of *H-2^b* molecules must be of a similar nature on both HFL/b and HFL/bk cells, and the modification of *H-2^k* molecules must be similar on both HFL/k and HFL/bk cells although determinants on modified *H-2^b* and *H-2^k* molecules are not recognised as cross reacting

Table 2 Selective killing of ^{51}Cr-labelled target cells by PEC from mice immunised with syngeneic or semi-syngeneic FV-induced tumour cells

Mice immunised	Tumour cells used for immunisation	% ^{51}Cr release from different target cells HFL/b	HFL/k	HFL/bk
BALB.K	HFL/k	3	10	66
BALB.B	HFL/b	59	10	46
(BALB B × BALB.K)F$_1$	HFL/k	4	55	60
(BALB B × BALB K)F$_1$	HFL/b	20	−5	

Methods as for Table 1.

antigens by the F_1 hosts. In spite of the possible occurrence of altered *H-2* molecules on these cells, they continue to express high levels of unaltered *H-2* specificities, as shown by their capacity to absorb antibodies to private *H-2* specificities expressed on uninfected cells (unpublished data). Thus, if the alteration of the *H-2* molecule is so extensive as to involve most of its antigenic features, then only a small proportion of *H-2* molecules can be so altered; otherwise the alteration in *H-2* must affect only a minor portion of the molecule.

We thank Philippe Roe for technical assistance and Dr Barry R Bloom for discussions. This work was supported by a contract with the Virus-Cancer Program of the National Cancer Institute. K.J.B. is supported by a National Institutes of Health training grant in genetics.

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Received December 5, 1975; accepted January 23, 1976.

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Demonstration of sensory neurones in the ectopic cuticle of spineless-aristapedia, a homoeotic mutant of *Drosophila*

MANY mutations in *Drosophila melanogaster* cause the development of body parts in inappropriate places. Several of these homoeotic mutations (reviewed in ref. 1) result in the appearance of leg bristles and other leg cuticular structures in place of their antennal counterparts. In the mutant spineless-aristapedia (ss^a, 3-58.5) the antennal arista is replaced by tarsal segments[2]; the mutations Antennapedia (*Antp*, 3-48) and Nasobemia (*Ns*, 3-48) produce a more extreme transformation: most of the antennal cuticular structures are transformed into leg cuticle, and, in the latter, part of the head cuticle is replaced by sternopleural cuticle[3,4]. In insects the sensory neurones are derived from epidermal cells[5]. When sensory epidermis is surgically transplanted to ectopic sites, neurones develop from it and form connections with the central nervous system[6]. These neurones act according to the type of epidermis from which they are derived rather than according to the position in which they develop[6]. In homoeotic mutants cuticular structures develop at ectopic sites as a result of genetic modification In this report I examine whether in homoeotic mutants sensory neurones are formed, and, if so, whether they behave according to the homoeotic structures that develop, or according to their general position in the body.

Antennae of Canton-S (C-S) and ss^a flies were examined histologically by three methods. In the first, half-heads of C-S and ss^a flies were fixed in glutaraldehyde and embedded in Epon, and sections 1 μm thick were cut and stained with methylene blue. In the second, flies' heads were fixed in Bouin's fluid, stained by the Bielchowsky silver technique, embedded in Epon and sectioned at 2.5 μm. Finally, flies were frozen and sectioned at 8 μm with a cryotome, and the sections were fixed and stained for acetylcholinesterase by the Karnovsky–Roots technique. The antennal tarsi of ss^a flies bear bristles, at the base of which can be seen nerve cells. By taking serial sections it was possible to show that processes extend from these cells and join to form a small bundle of fibres, containing acteylcholinesterase, that passes proximally through the tarsal segments into the second antennal segment where it joins the antennal nerve, which runs into the brain. The first and second antennal segments of ss^a flies seem normal, and the Johnston's organ is indistinguishable from that in antennae of normal flies. No muscles were found in the ss^a tarsus.

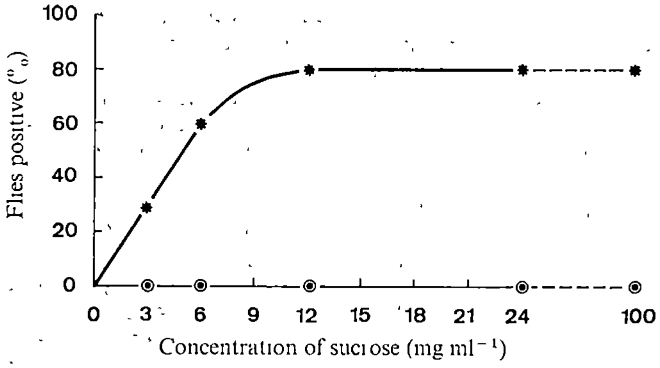

Fig. 1. Proportion of flies showing a proboscis-extension response when cuticular structures were stimulated with sucrose solutions of various concentrations. *, C–S legs; ⊙, C–S antennae. Each point is based on a minimum of 40 flies. The order in which the different appendages were treated with different solutions was varied randomly for each fly. For example, after being starved, attached to a slide, and water satiated (see text for details) a fly was tested as follows: five trials on tarsus of first left leg with sucrose (12.5 mg ml^{-1}), 10-min wait, during which the tarsus was washed, and the fly water satiated, five trials on left antennal tarsus with sucrose (6 mg ml^{-1}), 10-min wait, as above, five trials on tarsus of first right leg with sucrose (12.5 mg ml^{-1}) containing NaCl (150 mg ml^{-1}) and so on, for about 10 tests.

Histological examination, therefore, shows that neurones develop from homoeotic cuticle. A simple behavioural test was used to see whether such neurones are sensory, and whether they behave like receptors in leg structures. When the tarsal chemoreceptors of a normal hungry fly are stimulated with sucrose solution, the fly extends its proboscis[7]. Stimulation of antennae with the same solution elicits no response. This behavioural test (the proboscis-extension response) can be used to establish the presence in the cuticle of gustatory chemoreceptors the stimulation of which results in proboscis extension.

Male and female flies (5–10 d old) of the different genotypes were kept for 18 h in vials without food but containing moist filter paper They were then anaesthetised with carbon dioxide and attached, ventral side up, to a microscope slide, by a small drop of myristic acid (Baker) applied with a warm soldering iron. They were then left for 2 h to recover from the anaesthesia.

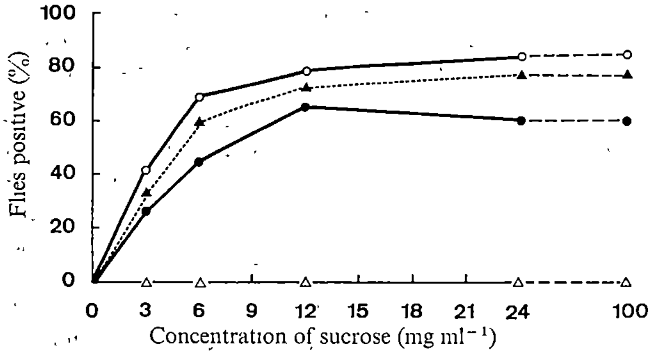

Fig. 2 Proportion of flies showing a proboscis-extension response when cuticular structures were stimulated with sucrose solutions of various concentrations. ○, ss^a legs; ●, ss^a aristal tarsi; ▲, *Antp*^{B} legs; △, *Antp*^{B} antennal legs. Each point is based on a minimum of 40 flies.

All flies were satiated with water before they were tested with sugar solutions. A droplet of water on the tip of an eyebrow hair was applied to one of the fly's tarsi. If the proboscis was extended in response, droplets were placed on the labellum and the fly was allowed to drink until further stimulation of the tarsus resulted in no extension of the proboscis—the fly then was water satiated. If this was not done the flies extended their probosces in response to water alone, which interfered with measurement of the dose–response curve for stimulation with sugar.

Presentation of a solution to a fly can modify its response to a second solution by changing the central excitatory state of the fly[8]. In C-S flies the response returns to normal within a few minutes (personal communication from Ghysen). To ensure that the central excitatory state was not influencing the flies' response, each was tested at 10-min intervals with solutions of different composition and concentrations, presented in random order. During each 10 min the test area of cuticle was washed with water to remove traces of the solution previously applied, and the fly was permitted to drink by placing a droplet of water on its labellum to ensure that it remained water satiated. Each test consisted of five consecutive applications at approximately 20-s intervals of the test solution to one of the test areas (that is, one of the leg tarsi, one of the antennae, or one of the antennal legs). A test was scored as positive when it resulted in extension of the proboscis twice or more in the five trials.

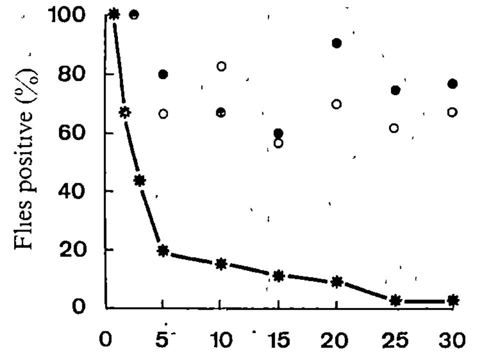

Fig. 3 Effect of NaCl on the response of tarsi to sucrose (12.5 mg ml^{-1}). *, C–S legs; ○, ss^a legs; ●, ss^a aristal tarsi. Only those flies showing a positive response to sucrose (12.5 mg ml^{-1}) were assayed; each point is based on a minimum of 20 flies.

The proboscis-extension response elicited when tarsi of the first legs of C-S flies were presented with sucrose solutions of various concentration is shown in Fig. 1. A maximum response (80%) was obtained with sucrose at a concentration of 12.5 mg ml^{-1}. Touching the antennae of these flies with the same solutions did not elicit a response (Fig. 1). About 20% of the flies did not respond to sugar at all; most of these were very inactive, and some died during the experiment.

Figure 2 shows that when the leg tarsi of ss^a flies were similarly stimulated the response was the same as that for C-S flies. When homoeotic antennal tarsi were stimulated a similar but weaker response was obtained (Fig. 2). A smaller proportion (60%) of flies showed the response when their antennal tarsi were stimulated than when their leg tarsi were stimulated (80%), and those flies that responded did so less rapidly to stimulation of their homoeotic tarsi than of their leg tarsi. Most of the 20% of flies that did respond to leg tarsal stimulation were also not stimulated by application of sugars to their antennal tarsi. It is not clear why of the 80% of flies that did respond normally to stimulation of their leg tarsi one quarter were unresponsive when their homoeotic tarsi were stimulated.

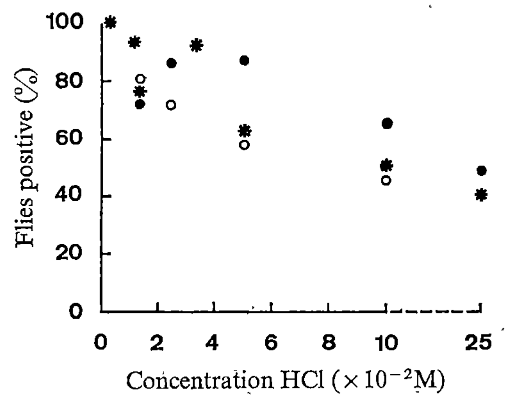

Fig. 4 Effect of HCl on the response of tarsi to sucrose (12.5 mg ml^{-1}). *, C–S legs; ○ ss^a legs; ● ss^a aristal tarsi. Only those flies showing a positive response to sucrose were assayed; each point is based on a minimum of 20 flies.

To establish whether the tarsal chemoreceptors in the antennae of ss^a flies respond with the same specificity as those in the legs, several sugars were tested by the same procedure. Only those flies were tested that showed a proboscis-extension response when their tarsi were tested with 12.5 mg ml^{-1} sucrose. Table 1 shows that the specificity of response of the antennal tarsi in ss^a flies was the same for these sugars as that of leg tarsi in C-S and ss^a flies, that is, the homoeotic tarsus was responding to these sugars as if it were a normal leg tarsus.

Sodium chloride and other salts added to the sugar solution inhibit the proboscis-extension response[9]. Figure 3 shows the effect of sodium chloride on the response of flies to sucrose. The tarsi of C-S flies were sensitive to sodium chloride, while those of ss^a flies were relatively insensitive. This was true both for the normal and for the antennal tarsi of ss^a flies, showing that, although this mutant is abnormal with respect to its response to sodium chloride, the ss^a antennal tarsi still behave exactly like ss^a leg tarsi.

In *Phormia regina* hydrochloric acid added to the sugar solution also inhibits extension of the proboscis[9]. The same is partially true for *Drosophila melanogaster;* in the case of this inhibitor ss^a leg and antennal tarsi responded to the same extent as C-S tarsi (Fig. 4).

In the mutants *Antp*^{B} and *Ns* the antennae are largely transformed into leg structures[3,4]. In both mutants the second and third antennal segments are partially or totally replaced by trochanteral, femoral and tibial structures[10], while in *Ns* the first antennal segment includes more proximal leg structures, and the head bears sternopleural cuticle.

Table 1 Specificity of response of tarsi

Genotype	Structure tested	Sugar (100 mg ml^{-1}) Melibose	Mannose	Fructose	Trehalose
C–S	Tarsus of first leg	19/53(36)	23/25(92)	25/25(100)	64/98(65)
	Antenna	0/50(0)	0/25(0)	0/25(0)	0/95(0)
ss^a	Tarsus of first leg	10/22(45)	19/19(100)	18/18(100)	21/42(50)
	Antennal tarsus	9/19(47)	14/15(93)	17/17(100)	19/40(47)

The figures given are the number of flies showing a positive response (that is, two or more proboscis extensions in five trials with the test sugar on the test area) out of the total number of flies tested with each sugar (percentages in parentheses).

Histological examination of femoral and tibial segments of the homoeotic legs showed that the bristles also have nerve cells at their bases. Axons from these cells run together through the leg and antennal segments to the antennal nerve, which passes into the brain. In $Antp^B$ flies the Johnston's organ is markedly reduced in size; in *Ns* flies this is even more extreme. No muscles were found in the antennal legs of $Antp^B$ flies; in *Ns* flies, muscles were only found in proximal homoeotic structures that correspond to the first antennal segment. In normal flies only the first antennal segment contains muscle; this perhaps explains why muscles are not found in the transformed second and third segments even though muscles would normally be present in the femur and tibia of the leg.

Stimulation of the antennal legs of $Antp^B$ flies did not elicit extension of the proboscis, whereas stimulation of the tarsi of the legs produced a response (Fig. 2). The same was true for *Ns* flies. This is expected since in neither stock of these mutants were tarsi present on the antennal legs. Unlike that of ss^a flies, the suger-elicited proboscis-extension response of the leg tarsi of $Antp^B$ and *Ns* flies was inhibited by salt at the concentrations that inhibit the response of normal flies. Hydrochloric acid also had the same effect on these mutants as on normal flies.

These results show that in the homoeotic mutation ss^a, which results in ectopic development of tarsal cuticle, a sensory apparatus is produced that can transmit information which can be interpreted correctly and used by the central nervous system of the fly. This ectopic sensory structure acts according to the type of epidermis from which it is derived rather than according to the position in the body in which it develops. This finding confirms, by genetic methods, the results of surgical transplantation experiments such as those done with the cricket, in which transplantation of sensory structures to ectopic sites resulted in the formation of functional sensory neurones[6].

This work was done while I was a Gosney Research Fellow of the California Institute of Technology, and was supported by a US National Science Foundation grant to Dr Seymour Benzer. I thank Dr Benzer for advice and support.

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Received November 12, 1975; accepted January 30, 1976.

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EEG α asymmetry and visuospatial performance

THE discovery of complementary cognitive specialisation of the two cerebral hemispheres has led to speculation about its significance for understanding individual differences in cognitive style. It has been suggested[1,2] that some people rely primarily on the left hemisphere whereas others rely on the right, and that this may be an important determinant of a person's cognition of his world.

Electroencephalographic (EEG) results have demonstrated that differential activation of the right or left hemisphere can be observed by analysing EEG α-band activity (8–12 Hz) during performance of appropriate cognitive activities. Since α waves are usually taken to be an indicator of cortical deactivation[3], relatively smaller α amplitudes in one hemisphere would presumably occur when that hemisphere is more active. A number of studies[4–10] have found changes in the relative amounts of α activity in the two hemispheres which depend on the type of cognitive tasks a subject performs. The direction of this asymmetry is predictable from the known cognitive capabilities of each hemisphere. Linguistic tasks, such as composing a letter or mental arithmetic, show relatively more right deactivation. Similarly, visuospatial and musical tasks, which have been linked with right-hemisphere capabilities, show relatively more left deactivation.

If the suggestion that there are individual differences in hemispheric usage is correct, then it would be expected that persons who scored highest in objective tests of visuospatial abilities would have the lowest ratios of right-hemisphere α to left-hemisphere α. We tested this hypothesis by recording right and left posterior EEGs while subjects solved problems involving the imaginal manipulation of visually-presented forms. We have found that subjects with relatively less activity (and consequently more activation) in the right hemisphere perform more efficiently tasks which are known to rely on the right hemisphere.

Subjects were 16 right-handed students, recruited from an introductory course in psychology at Dartmouth College. The test of visuospatial imagery consisted of a series of 20 problems, selected from three different sources. Two types of problem were adapted from chronometric studies of visual imagery by Shepard and coworkers[11,12], and the third type was taken from a standardised test of spatial ability[13]. The three problem types were similar to each other in requiring the imaginary manipulation of objects in space, an ability shown to be preferentially impaired by right-posterior cortical lesions[14,15] and performed poorly by the left hemisphere of commissurotomised patients[16,17]. Figure 1 illustrates these problems.

The series of test items was presented to each subject in the same fixed order, in three blocks of trials separated by 2-min rest periods. Each problem was mounted on a card (5 × 8 inches) which the subject held in his hands during the test. Subjects were instructed to answer each problem verbally as soon as the answer was known, and to avoid double-checking their answers before responding. They were also instructed to keep their eyes open and to look at the card while solving the problem. Answers and time of response were recorded on the EEG polygraph protocols, and latencies were estimated to the nearest 0.5 s from the protocols.

Two EEG channels were recorded while the subject worked on the problems, and also during a 30-s eyes-open baseline before the problem series. The EEG channels were derived from homologous occipitoparietal sites: one at the midpoint between O_1 and P_3; the other halfway between O_2 and P_4. Each of these was referenced to its ipsilateral earlobe. The EEGs were recorded conventionally with Grass gold-cup electrodes, Grass 7P511 amplifiers, and an Ampex PR500 tape recorder.

The recorded EEG signals were subsequently played back through matched α-bandpass filters (half-amplitude points at 8.5 and 12 Hz), and the output of the filters was integrated by circuits which reset every second. Two polygraph pens were driven by the integrators, and α amplitudes for each channel were measured by the distance the pens traversed on being reset. The output of this system was linear with the amplitude of a 10-Hz sine wave in the range of amplitude values used in this study.

Ratios of integrated α activity from the right hemisphere to α activity in the left hemisphere (R/L ratios) were computed for each 1-s epoch. A rank order plot of overall average ratios against average time to problem solution for each subject is shown in Fig. 2, indicating that those subjects with the lower R/L ratios solved the problems most efficiently. This result is in the direction predicted by the hypothesis of Bogen *et al.*[1] and is consistent with previous studies of EEG asymmetry[4–10]. Subjects who have relatively less right-α activity and therefore relatively more right activation, perform better on a putative right hemisphere task.

The correlation found here is nearly as strong if R/L ratios recorded during the resting baseline period are correlated with the average problem–solution latencies ($\rho = 0.508$, $P<0.05$). Apparently, most of the correlation observed in these data is a product of a tonic asymmetry in the EEG rather than a momentary activation pattern.

Dumas and Morgan[6] failed to find a relationship of the type reported here. These investigators used occupational category as the criterion variable for cognitive ability, arguing that a group of engineers would be expected to exhibit higher R/L α ratios than a group of artists. One difficulty with this research strategy is that there may be too many variables other than cognitive predisposition which determine occupational choice. Correspondingly, there may be, within any one occupation, great variety in the ways in which one can approach a task. For example, it is frequently observed that some mathematicians are clearly geometers—that is, they think about mathematics in visuospatial terms, and their proofs tend to be based on geometrical conceptions. There are also other mathematicians who are said to be algebraists, who tend to think mathematically in terms of the propositional manipulation of symbols. Similar kinds of differences are likely to be found within any occupational category.

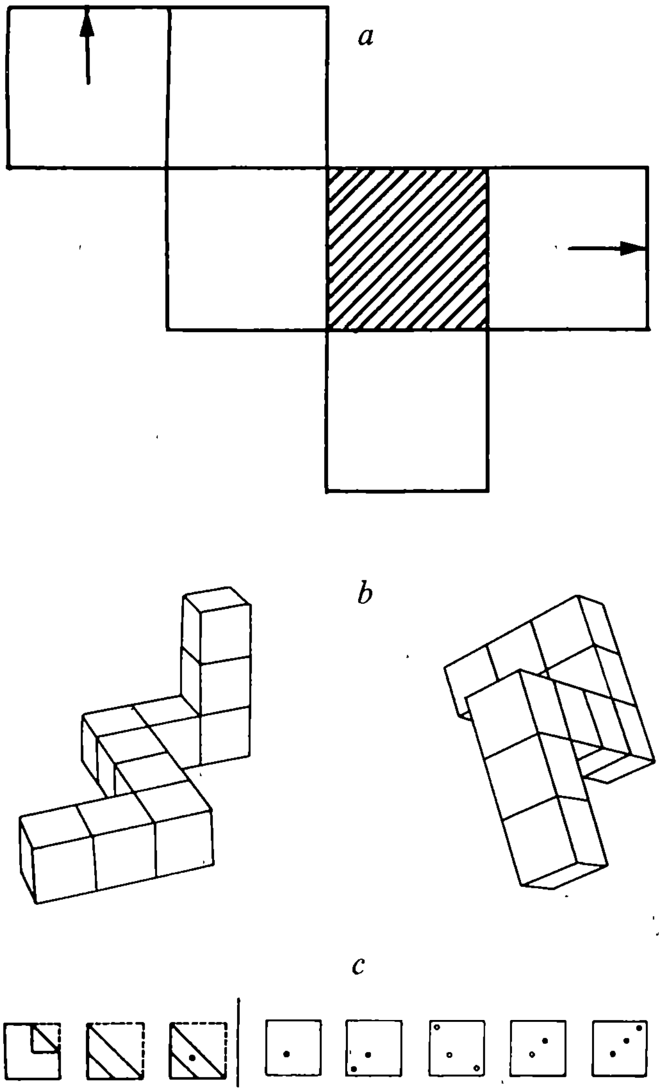

Fig. 1 Examples of the three types of visuospatial problems. *a*, Cube folding: mentally fold the figure into a cube, using the striped side as the base, and decide whether or not the two arrows meet (after ref. 12). *b*, Three-dimensional rotation: rotate in space one of the two figures and decide whether they are congruent or mirror opposites (after ref. 11). *c*, Paper folding: observe the sequence of folding operations in the left figures and decide the outcome of punching the indicated hole by choosing the correct figure on the right (from ref. 13).

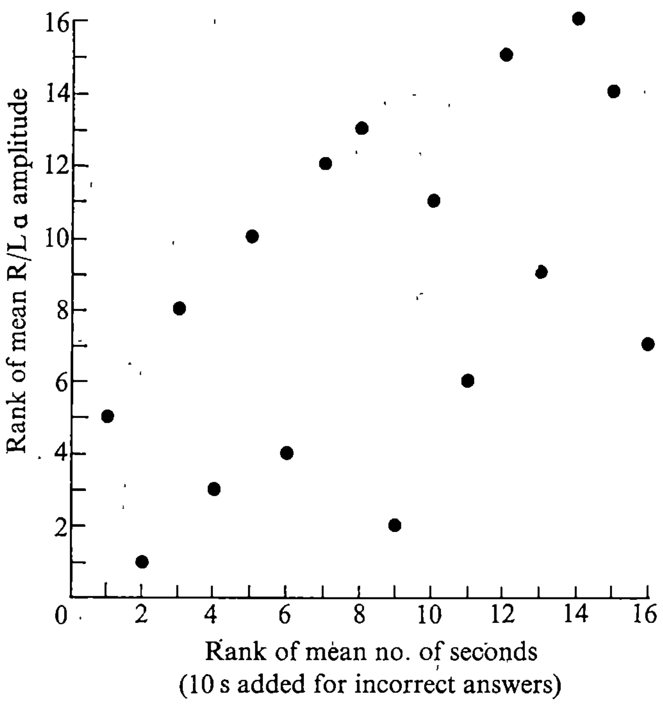

Fig. 2 Rank order scatter plot of average EEG asymmetry against average problem–solution latency. Ratios of right-to left-α activity were computed for each 1-s epoch and averaged over each trial for each subject. Values shown here are subject averages over all problems. Rank data are given because of the skewness of the distribution of average latencies. These data produce a rank order correlation of 0.546 ($t = 2.44$, d.f. $= 14$, $P < 0.05$). The latencies ranked here have been corrected for errors by adding 10 s to the time to solution on incorrect trials. If no correction is made for incorrect answers, the value of the correlation is attenuated only slightly ($\rho = 0.515$, $P < 0.05$).

The subjects tested in the present investigation, being sampled from a group of bright college students, were no doubt selected in part for visuospatial ability. It is a well known statistical fact that such a restriction in the range of one variable tends to reduce the value of the correlation coefficient[18], indicating that the present result might underestimate the correlation in the larger population.

I thank Sue Thompson for assistance, and John Polich and Gregory McHugo for valuable comments. This research was supported by the National Institute of Child Health and Human Development.

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Received November 6, 1975, accepted January 2, 1976.

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Bicuculline reversal of deprivation amblyopia in the cat

CATS deprived of visual experience in one eye during a critical period of early life develop marked changes in the functional organisation of their visual system[1-3]. Useful vision seems to be lost in the deprived eye, and neurophysiological studies reveal a loss of binocular input to neurones in the visual cortex. Most neurones fail to respond to stimuli presented to the deprived eye; whereas almost all respond to stimulation of the normal eye. In normal neonatal kittens without visual experience, most cortical neurones respond to stimulation of either eye. Moreover, this binocular neuronal input persists when subsequent visual experience is prevented by binocular lid suture. Thus, asymmetrical visual experience seems necessary to produce a loss of binocular input. This loss of functional input from the deprived eye could result from a loss of the normal excitatory synaptic connections or from inhibitory suppression of afferent input. We postulate that asymmetrical visual experience causes synaptic inhibition of input from the deprived eye and that reduction of such inhibition might restore binocularity.

Several lines of evidence suggest that inhibition in the cat visual system may be mediated by γ-aminobutyric acid (GABA). Of particular importance are the reports that bicuculline, a GABA-receptor blocker[4], is able to alter receptive field (RF) properties of normal visual neurones[5-8]. We, therefore, attempted to restore binocularity in monocularly deprived cats by intravenous administration of bicuculline. This drug was able to restore binocular input to more than half the cortical neurones tested.

Five kittens were monocularly deprived of vision by lid suture in their fourth week of life. After 8 months of monocular deprivation, extracellular single unit recordings were made in the area centralis of visual cortex. We used paralysed (gallamine triethiodide) and locally anaesthetised (xylocaine) preparations to avoid possible confounding of our results by the known neurophysiological effects of general anaesthesia[9]. Single-cell activity, electroencephalogram (EEG), stimulus marker, and a voice channel were tape recorded. Each eye was independently stimulated with moving slits and bars of light projected on a tangent screen on which the eye was focused by a contact lens. An insulated tungsten microelectrode was advanced while stimulating the normal eye. RFs of responsive cells were characterised as "simple, complex, or hypercomplex"[10,11]. Next, the amblyopic eye was stimulated before and after the administration of intravenous bicuculline, usually given in 0.1–0.2-mg aliquots (0.03–0.06 mg kg^{-1}).

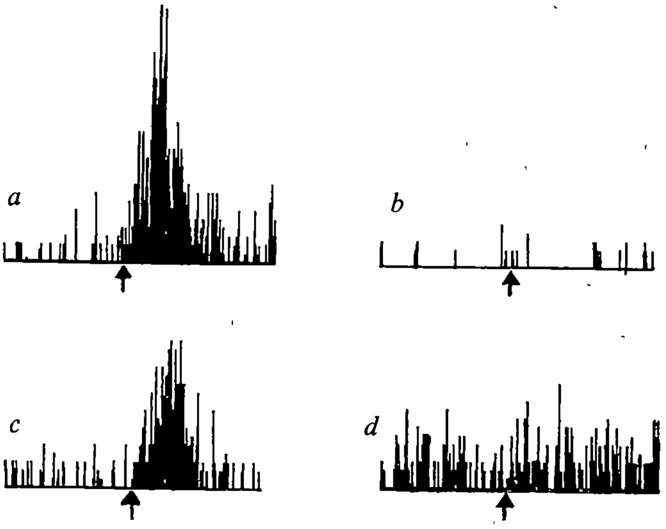

Fig. 1 Stimulus-related response histograms for monocular stimulation ($n = 10$) of a single cell in cat visual cortex with a complex RF. Time base is 512 ms Arrow indicates entry of light-bar stimulus into the receptive field of the cell. Same stimulus parameters (size, shape, orientation, direction and speed) used for each histogram. *a*, Normal eye open, pre-drug; *b*, amblyopic eye open, pre-drug: no response; *c*, amblyopic eye open, 2 min after 0.06 mg kg^{-1} of intravenous bicuculline: note response to stimulation and its similarity to *a*; *d*, amblyopic eye open, 15 min after bicuculline: note disappearance of response in spite of higher background firing rate. 20 of 33 cells developed similar responsiveness to stimulation of amblyopic eye after bicuculline which was independent of changes in background firing rate.

Table 1 Response of neurones to bicuculline by RF type (responsive cells/total cells tested)

Cat	All cells	Simple	Complex	Hyper-complex
1	1/1	1/1	0/0	0/0
2	4/8	3/4	2/4	0/0
3	6/10	1/1	3/7	2/2
4	5/10	2/3	2/5	1/1
5	4/4	0/0	3/3	1/1
All:	20/33	7/9	10/19	4/4
	(60.61%)	(77.78%)	(52.63%)	(100%)

All 33 cells evaluated responded to stimulation of the normal eye and had normal RF patterns. In contrast, only one could be driven by the amblyopic eye. Few unresponsive cells were encountered. These results agree with other neurophysiological studies of monocular deprivation amblyopia[1-3].

Administration of intravenous bicuculline produced a striking change in more than half the cells studied. Nineteen cells now responded to stimulation of the amblyopic eye (Figs 1 and 2). Although the brief duration (Fig. 2) of the response limited our study, the new amblyopic receptive field of a given neurone seemed similar, if not identical, with respect to location, size, shape, orientation specificity and directional specificity to the RF found with stimulation of the normal eye Cells of each RF type (simple, complex, hypercomplex) showed a response to bicuculline (Table 1). The amblyopic RF tended to show wider orientation tuning[12] than the pre-drug RFs to stimulation of the normal eye; however, most normal eye RFs showed a similar widening of their orientation tuning after bicuculline. The one cell responsive to stimulation of the deprived eye before bicuculline, which had shown an abnormal RF type, developed a more normal RF after bicuculline. Thus, relatively normal RFs to stimulation of the deprived eye were brought out in 20 of the 33 cells studied (60%). The remaining 13 cells (40%) failed to respond to stimulation of the amblyopic eye in spite of sufficient bicuculline to produce seizure discharges on EEG. The responses of 17 cells began within 30 s of bicuculline administration and lasted up to 10 min. The remaining 3 cells had a delayed response onset and a longer duration, lasting up to 30 min.

The administration of bicuculline was often complicated by its potent convulsive effects. Seizure discharges lasting 2–3 min often followed the bicuculline response within 15–30 s. We do not feel, however, that the restoration of binocularity was due to some nonspecific increase in excitability associated with seizure activity. All neurones which responded to stimulation of the deprived eye did so at subconvulsive doses of bicuculline Moreover, the appearance of seizure discharges at higher dose levels was always associated with loss of any response from the amblyopic eye. Finally, in two cells which had previously responded to bicuculline, we found that intravenous physostigmine failed

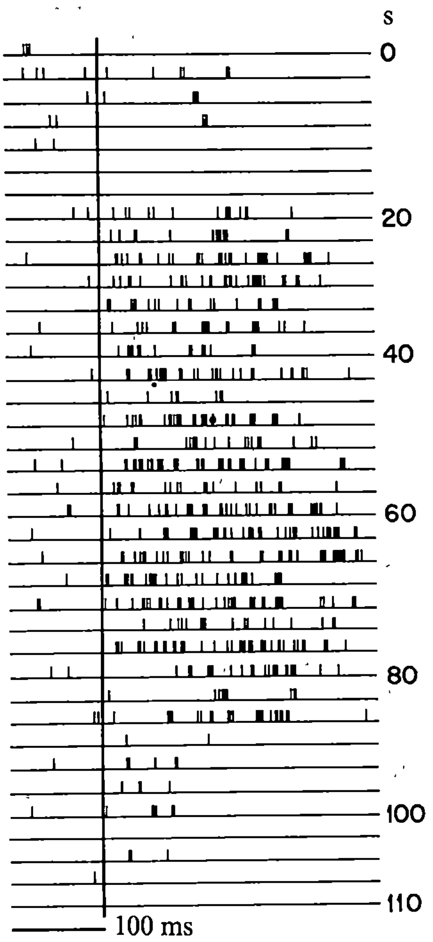

Fig. 2 Neuronal activity (Schmitt trigger output) of a complex visual cortical cell during monocular stimulation of the deprived eye. The continuous vertical line indicates entry of an optimally oriented light bar into the RF. Time base is 400 ms Responses of the cell to sequential stimuli are shown in temporal order from top to bottom. The first line is immediately after 0.03 mg kg^{-1} of intravenous bicuculline. Time (s) after bicuculline is shown to the right. Note the rapid onset of response at 20 s and its disappearance by 90–100 s. At higher dose levels, binocularity could be restored for longer periods of time.

to restore binocularity even at dose levels producing seizure activity.

Thus, our results indicate that intravenous bicuculline can make visual cortical neurones accessible to stimulation of the deprived eye in cats with monocular deprivation amblyopia. Access from the deprived eye could be restored after full development of the amblyopic condition with 8 months of visual deprivation. Moreover, the amblyopic eye RF showed the same properties as the normal eye RF. These observations suggest that the pathways between the amblyopic eye and the visual cortex may be capable of normal physiological function in spite of morphological changes which have been found in such preparations[13]. Since bicuculline was given intravenously, our data do not indicate where it acts to restore binocularity.

We postulate that the restoration of binocular input by bicuculline occurs by reduction of inhibitory mechanisms by way of GABA-receptor blockade. This mechanism is compatible with available information about the cat visual system. First, inhibitory input has been proposed as an important determinant of the normal response characteristics of visual neurones[14,15]. Second, GABA has been implicated as a visual inhibitory transmitter[16,17]. Finally, bicuculline alters the RFs of normal visual neurones in a manner consistent with reduction of inhibition[5–8]. If our interpretation is correct, then the total dominance by the normal eye results from active inhibition of the relatively intact input from the amblyopic eye and suggests that feline amblyopia may not involve a permanent, irreversible loss of visual function.

This work was supported by grants from the US National Institutes of Health.

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Dopamine release in substantia nigra?

IN addition to the axons which project to the nucleus caudatus[1,2], the dopamine (DA)-containing perikarya of the substantia nigra have extensive dendritic processes. These dendrites, observed after Golgi staining[3–5] and by electron microscopy[3,4], contain relatively large amounts of DA—revealed by histofluorescence[6]—and vesicle-like structures[4]. The DA seems to be stored in reserpine-sensitive structures[6]. The question has arisen whether DA can be released from the dendritic processes, as well as from nerve terminals of the nucleus caudatus[7,8]. Our investigation of the metabolism of released DA suggests that it can.

3,4-Dihydroxyphenylacetic acid (DOPAC) is the main metabolite of striatal and limbic DA and less than 50% of DOPAC is converted to homovanillic acid (HVA)[9]. We have investigated the metabolism of DA after electrically stimulated release in areas containing exclusively nerve terminals (nucleus caudatus[10], nucleus accumbens and the olfactory tubercle) and in a mesencephalic area devoid of nerve endings, but containing cell bodies (groups A_8, A_9 and A_{10})[1,11] and DA-rich dendrites[6] Rats (males, 200–230 g, Wistar-derived strain) were anaesthetised with chloral hydrate (400 mg kg^{-1}, intraperitoneally) and placed in a stereotaxic device A monopolar electrode was inserted through a burr hole in the exposed skull, with the coord-

Table 1 Alterations of striatal concentrations of DA, DOPAC and HVA after electrical stimulation of MFB

	Concentration in corpus striatum (μg g^{-1}±s.e.m)		
Compound	Ipsilateral	Contralateral	Difference
DA (7)	9.05±0.40	10.74±0 28	1 70±0.24
HVA (6)	2 54±0 16	1.77±0 09	0.77±0 15
DOPAC (9)	3.30±0.16	1.78±0 11	1.52±0.24

Stimulation was for 10 min, 30 Hz, 300 μA Number of experiments in parentheses. All differences between ipsilateral and contralateral sides $P < 0.005$.

Table 2 Alterations of the concentration of DOPAC in various areas in the brain after stimulation of MFB

Brain area (*N*)	Concentration of DOPAC±s.e.m. (µg wet weight) Ipsilateral	Contralateral	Difference
Antidromic activation			
Mesencephalic area including cell bodies +dendrites (11)	0.39±0.02	0.26±0.01	0.13±0.02
Orthodromic activation			
Olfactory tubercle (10)	3.35±0.14	1.83±0.11	1.51±0.14
Nucleus accumbens (6)	3.31±0.21	2.27±0.19	1.04±0.20
Corpus striatum (9)	3.30±0.16	1.78±0.11	1.52±0.24

N, Number of observations (in parentheses). All differences between ipsi- and contralateral sides are significant at $P < 0.005$. Stimulus parameters: 300 µA; biphasic; frequency 30 Hz; duration 10 min

inates as described before[8]. The tip of the electrode was placed in the median forebrain bundle (MFB) which was stimulated electrically for 10 min (biphasic constant current of 300 μA; frequency 30 Hz; pulse length 2 ms). These stimulation parameters were found to give a maximal release of DA, and subsequent formation of DOPAC and HVA[8,12]. After stimulation we dissected various brain areas, including the nucleus caudatus, nucleus accumbens, olfactory tubercle and 12 mg of mesencephalic tissue containing both the substantia nigra (catecholamine groups A_8 and A_9) and group A_{10} of the ventral tegmental area, as described before[8,9,13,14].

In all cases the concentrations of the metabolites of DA in areas ipsilateral to the electrode were compared with those in the corresponding contralateral areas. DOPAC, HVA and DA were analysed as before[8,13,14].

Within 10 min of stimulation striatal DA was decreased by about 20%, while the concentration of DOPAC and HVA had increased (Table 1) The rise of the concentration of DOPAC was about double that of HVA, indicating that DA after release is predominantly deaminated. In these stimulatory conditions the potent inhibitor of DA uptake, nomifensine, had no effects on the metabolism of DA, demonstrating that there is no reuptake of released amine[8]. In all areas studied, DOPAC was increased by electrical stimulation of the MFB, thus including the non-terminal areas of the brainstem, which are antidromically activated (Table 2) These observations strongly suggest that when a nerve impulse is generated in dopaminergic neurones, DA is released not only from nerve terminals, but also from the cell bodies and dendrites. In addition we found (unpublished) that various drugs, including morphine, oxotremorine and neuroleptics, increase the concentrations of DOPAC and HVA not only in striatal and limbic structures, but also in the mesencephalic area containing dopaminergic cell bodies and dendrites.

What function can be attributed to DA released from non-terminal structures? We shall consider three possibilities First, DA may inhibit the neurone from which it is released (self- or auto-inhibition). Bunney and Aghajanian[15] found that DA-containing neurones of the substantia nigra were inhibited when the amine was applied microiontophoretically in the vicinity on the perikarya. With this proposed mechanism dopaminergic neurones may restrict the range of their firing frequencies. Second, DA released in the substantia nigra may inhibit other neurones, located near the dendrites releasing the amine (lateral inhibition). Such mechanisms have been described in other areas in the central nervous system, including the retina. Dendro-dendritic contacts in the substantia nigra have been revealed by electron microscopy[4] Groves *et al.*[16] found that local infusion of amphetamine, a drug which causes release of DA, also inhibits neuronal activity in the substantia nigra. This finding, however, does not discriminate between the two hypotheses.

The third possibility is that dopaminergic neurones have no preferential orientation Thus the observed dendrites can be considered to have the same function as the aminergic varicosities observed in the other parts of the brain. Neurones without obvious polarity include the retinal amacrine cells, of which a subpopulation seems to use DA as a neurotransmitter[17]. Which of these possibilities, or combination of possibilities, is valid for dopaminergic cells of the substantia nigra cannot be decided from available knowledge, including the data reported here. Our data do show that released DA is rapidly converted to DOPAC, not only in areas of the brain containing nerve terminals, but also where only perikarya and dendrites are found. These results challenge the concept that DOPAC is formed exclusively intraneuronally and that release of a neurotransmitter occurs only from nerve terminals.

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Release of dopamine from dendrites in rat substantia nigra

THERE is increasing evidence that neurotransmitter synthesis, storage and release are not confined to axon terminals Much of this evidence has come from studies of the dopaminergic neurones of the substantia nigra (see Fig. 1). The terminal dendritic processes of dopamine (DA) neurones in substantia nigra are varicose and contain clusters of small vesicles[1,2]; histochemical studies have shown that the dendrites contain DA and are able to take it up[3,4]. There is also evidence that the components necessary for the synthesis and storage of DA are transported

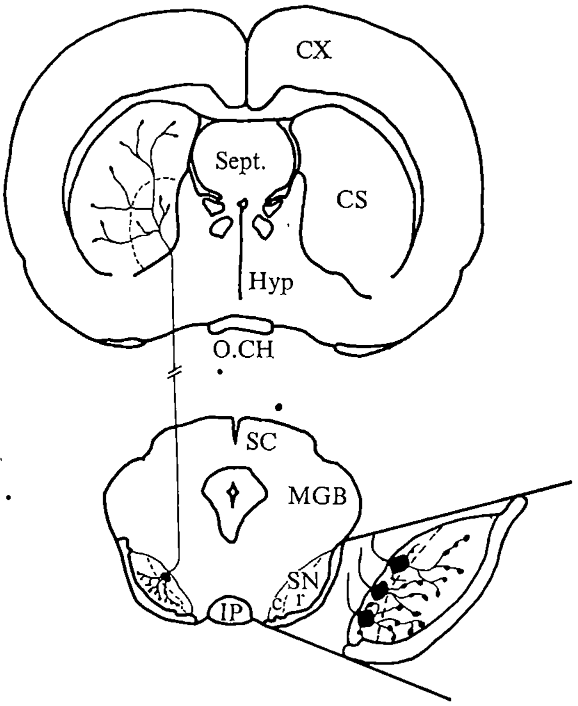

Fig. 1 Schematic representation of the dopaminergic nigrostriatal pathway. The cell bodies of dopaminergic neurones are largely confined to the pars compacta of substantia nigra with a well defined anatomical segregation of their axonal and dendritic processes[1]. Within substantia nigra, the dendrites of these neurones radiate ventrolaterally into the pars reticulata, whereas the axons project rostromedially ending mainly in the corpus striatum. Some dendrites are also present in the pars compacta. CX, Cerebral cortex; CS, corpus striatum; Sept., septum pellucidum; Hyp., hypothalamus; OCh, optic chiasma; SC, superior colliculi; MGB, medial geniculate body; IP, nucleus interpeduncularis; SN, substantia nigra; c, pars compacta of the substantia nigra; r, pars reticulata of the substantia nigra.

somatofugally in dendrites of substantia nigra[3,5]. Finally, the local application of DA agonists to the substantia nigra produces an inhibition of firing of neurones in the pars compacta that is blocked by DA antagonists[6,7]. On the basis of these observations, it has been proposed that a local dendritic release of DA may modify the excitability of dopaminergic neurones in substantia nigra[7]. There has, however, been no direct demonstration of transmitter release from these or other dendrites. In this study we present evidence for the release of DA from dendrites of dopaminergic neurones in rat substantia nigra.

We have used a combination of methods that provides a refinement of previous techniques for the study of the release of neurotransmitters in the central nervous system (CNS), enabling separate superfusion of fresh, microdissected areas of rat brain rich in cell bodies, dendrites or axon terminals of the same neurones.

The entire substantia nigra and samples of corpus striatum were dissected from serial slices (300 μm) of rat brain using the method of Zigmond and Ben Ari[8]. The pieces of tissue were incubated in ^{3}H-DA and uptake of labelled amine was measured after a 15-min incubation (Table 1). The endogenous DA content of similar samples was measured by a radiochemical enzymatic method[9]. ^{3}H-DA uptake per unit wet weight of tissue was 57% higher in corpus striatum than in substantia nigra, whereas the striatal DA concentration was more than sevenfold higher. Expressed in terms of endogenous DA concentration, therefore, ^{3}H-DA uptake and retention was greater in substantia nigra than in corpus striatum. One explanation of this finding is that vesicles in dendrites may have a lower endogenous transmitter content and a higher capacity for retention of exogenous DA than vesicles in axon terminals. In sympathetic neurones, axonal vesicles have been shown to increase their noradrenaline content fourfold on migration down the axon[10], with a concomitant decrease in capacity for uptake and retention of exogenous noradrenaline[11].

The evoked release of neurotransmitters from axon terminals in CNS by depolarising potassium concentrations has been extensively studied *in vitro*[12,13]. Slices of substantia nigra and corpus striatum were labelled with ^{3}H-DA and superfused with Krebs bicarbonate solution. The spontaneous release of ^{3}H-DA was initially rapid but became stable after 20 min superfusion. Brief exposure to high potassium (23.5 mEq l^{-1}) evoked large and reproducible increases in ^{3}H-DA efflux from both substantia nigra and corpus striatum (Fig. 2*a* and *b*). Slices of substantia nigra released a significantly higher proportion of their ^{3}H-DA content than corpus striatum, both spontaneously and in response to potassium stimulation (Table 2). This may be related to the observation that the specific activity of ^{3}H-DA in substantia nigra was considerably greater than in corpus striatum (Table 1).

The spontaneous efflux of ^{3}H-DA from both tissues was unaffected by removal of calcium from the perfusion medium, but potassium-evoked release was reduced by 88±2% in substantia nigra and 85±6% in corpus striatum in the absence of calcium (mean±s.e.m. $n=4$) (Fig. 2*c* and *d*). In each case, potassium-evoked release of ^{3}H-DA could be restored by a brief exposure to normal calcium. Raising the magnesium concentration in the presence of calcium also reduced the potassium-evoked release, by 59±1% in substantia nigra and by 72±10% in corpus striatum (mean±s.e.m., $n=4$) (Fig. 2*c* and *d*). There were no significant differences between substantia nigra and corpus striatum in the inhibitory effects of low calcium or high magnesium concentrations. Since the release of neurotransmitters by exocytosis is known to be calcium dependent and to be inhibited by high magnesium concentrations[14], it is likely that DA released from corpus striatum and substantia nigra was vesicular in origin.

Further evidence for vesicular release was obtained by estimating the proportion of radioactive metabolites of

Table 1 Dopamine uptake and content in rat substantia nigra and corpus striatum

	Tissue wet weight (mg)	Endogenous DA (ng mg^{-1})	^{3}H-DA uptake (ng mg^{-1})	^{3}H-DA/endogenous DA ratio
Substantia nigra (cell bodies and dendrites)	3.11±0.22 (12)	0.34±0.02 (4)	1.22±0.10 (7)	3.59
Corpus striatum (axon terminals)	6.16±0.33 (10)	2.55±0.31 (4)	1.92±0.16 (7)	0.75

Adult male Wistar rats were anaesthetised and perfused through the left ventricle of the heart with 100 ml Krebs phosphate solution at 4 °C. Coronal slices (300 μm thick) of the brain were cut in a 4 °C cold room with a McIlwain tissue chopper, and the entire substantia nigra and a portion of corpus striatum at the level of the optic chiasma were dissected using a stereomicroscope. Tissue samples were weighed and preincubated in Krebs–Henseleit solution at 37 °C for 5 min ^{3}H-DA (specific activity 8.95 Ci mmol^{-1}; NEN Chemicals) was added to a final concentration of 1 μM and incubation continued for a further 15 min. After rinsing, the tissue radioactivity in acid methanol extracts of nigral and striatal tissue was shown by ascending chromatography (*n*-butanol–acetic acid–water, 4:1:1 v/v) to migrate with authentic DA. Each value is mean ± s.e.m. Number of samples in parentheses.

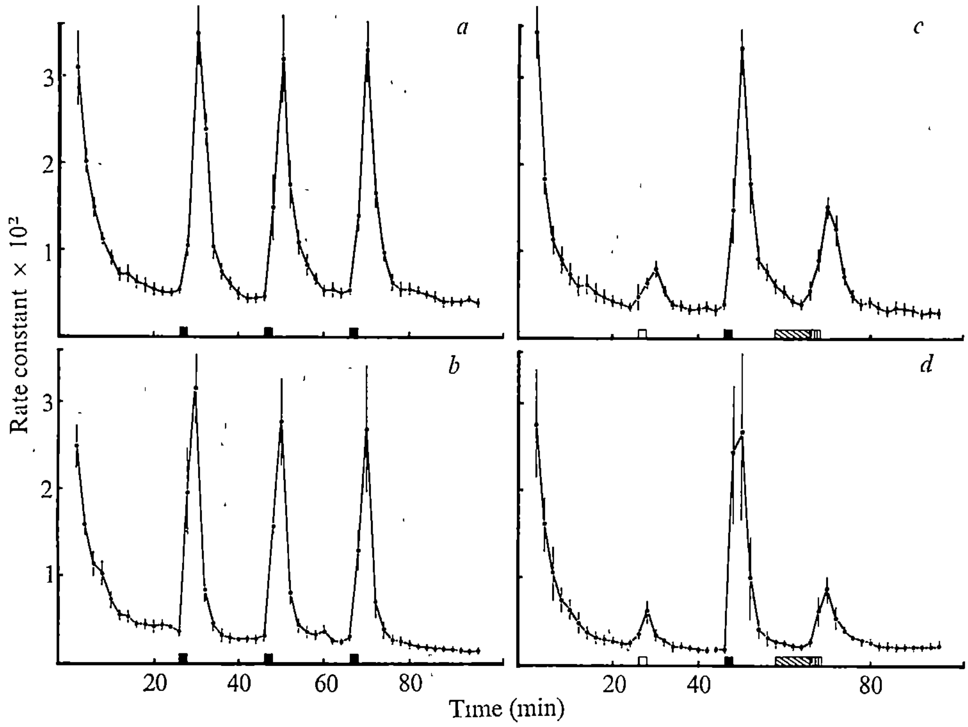

Fig. 2 Release of ^{3}H-DA from superfused slices of rat substantia nigra (*a*, *c*) and corpus striatum (*b* *d*). Tissues were superfused at a rate of 30 ml h^{-1} with normal Krebs bicarbonate solution. At 20-min intervals, pulses of high potassium (23.5 mEq l^{-1}) were given for 2 min. In *a* and *b*, potassium pulses were given during perfusion with normal Krebs (■); in *c* and *d*, superfusion throughout the experiment was with calcium-free Krebs. The first potassium pulse was applied in the continued absence of calcium (□). For the duration of the second (■) and third (vertical hatching) potassium pulses the calcium concentration was restored to normal. Four fractions before, and during the third potassium pulse, the magnesium concentration was raised to 24 mEq l^{-1} (diagonal hatching); after the third potassium pulse, the magnesium concentration was restored to normal. Efflux of radioactivity was expressed as the fractional rate constant (d.p.m. released per min in superfusate/d.p.m. in tissue stores at time of collection). Each value is mean ± s.e.m. of four experiments.

^{3}H-DA in the superfusates. During stimulation of sympathetic nerves it has been shown that the ratio of released noradrenaline to its metabolites increases greatly[15], presumably because transmitter released by exocytosis is not susceptible to intraneuronal metabolism. In some of the present experiments samples of superfusate were chromatographed on alumina columns[16]. The proportion of total radioactivity recovered in the catechol fraction was 60.1 ± 4.5% in basal samples and 90.5 ± 1.7% during the peak of the potassium-evoked release in both substantia nigra and corpus striatum (mean ± s.e.m., $n=4$).

To minimise the possible contribution to ^{3}H-DA release made by dopaminergic cell bodies in the substantia nigra, further experiments were carried out using only the pars reticulata of substantia nigra, removed by careful microdissection. Each potassium pulse released 7.8 ± 0.5% (mean ± s.e.m., $n=4$) of ^{3}H-DA stores in pars reticulata. Thus, although it is not yet possible to assess the magnitude of ^{3}H-DA release from neurone cell bodies in the pars compacta it is clear that a substantial proportion of the ^{3}H-DA release observed in experiments on the entire substantia nigra occurs from dendritic processes

Table 2 Fractional release rates of ^{3}H-DA from substantia nigra neurones

	Spontaneous	K$^+$ evoked
Substantia nigra (cell bodies and dendrites)	0.50±0.02	5.76±0.33
Corpus striatum (axon terminals)	0.32±0.03*	4.25±0.54†

Values for spontaneous efflux are expressed as the mean ± s.e.m., ($n=12$) percentage of tissue ^{3}H-DA stores released in the three fractions before each potassium pulse; for potassium-evoked release as the total increase in ^{3}H-DA release over basal level for each pulse. Significance of difference between substantia nigra and corpus striatum: $^*P < 0.001$; $^\dagger P < 0.05$.

The release of transmitter from dendrites may have a number of functions. Coordination of excitability of dopaminergic neurones within substantia nigra could be mediated by a DA receptor located either on the DA neurones or presynaptically on the axon terminals innervating them, or both. Release of transmitter close to cell bodies could also provide a mechanism for the local control of neuronal metabolism. A third regulatory function for the dendritic release of transmitter could be in the control of plastic and regenerative sprouting of neuronal processes and the establishment of new synapses.

We acknowledge a travel grant from the Wellcome Foundation to L.B.G. and an MRC Scholarship to T.M.J. We also thank Ms L. Mercer for assistance.

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Received December 5, 1975; accepted January 29, 1976.

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Foetal thymidine kinase in tumours and colonic flat mucosa of man

WE have characterised two forms of deoxythymidine kinase (TK) using antisera prepared against the enzyme partially purified from human colonic mucosa. One of these forms is associated with rapidly proliferating lower colonic crypt cells and the other, with non-dividing surface cells. Antibody to the latter cross reacted with several tumours and placental extracts but not with phytohaemagglutinin (PHA)-stimulated or leukaemic lymphocytes. This cross reactivity of surface cells and colonic tumours supports the concept that surface cells rather than crypt cells are the site of origin of colonic cancer[1,2].

Studies in preneoplastic tissues showed a persistent incorporation of labelled deoxythymidine into DNA of the normally quiescent cells of colonic[1–3] and gastric[4] mucosae and cervical epithelium[5]. These observations directed our attention to the enzymes associated with DNA synthesis in these non-dividing cells as well as normal and malignant proliferating tissues. The concomitant increases in DNA synthesis and the pyrimidine salvage enzyme, TK, in proliferating cells and tissues and physiologically stimulated systems[6,7] focused on this enzyme as a parameter linked to proliferation and malignancy. For example, human colorectal tumours contain more TK than the homologous, normally rapidly proliferating tissue[8]. In the latter, TK activity is found primarily in the lower third of the colonic crypt, the region containing most of the rapidly proliferating cells[3,8,9]. Tumour TK differs from the normal enzyme in its response to mercaptans and is more thermolabile in the presence and absence of mercaptans[8].

These data, as well as the observations that TKs induced by infection with herpesvirus types 1 and 2 are biochemically and antigenically distinct not only from cellular TK but also from each other[10,11], suggested that the serological properties of TK could be used to detect and characterise colorectal malignancies. Antisera were prepared in the rabbit, using partially purified TK from total normal colonic mucosa as immunising antigen (Table 1), and screened by Ouchterlony double diffusion technique[12] against cytosol (127,000g supernatant fraction) TKs from normal total colon, colonic surface and crypt cells, placenta, PHA-stimulated lymphocytes, leukaemic cells and tumours from sites other than the colon.

Both preimmune and immune sera were tested against homologous and heterologous enzymes. Preincubation with preimmune sera did not have any effect on TK activity of any

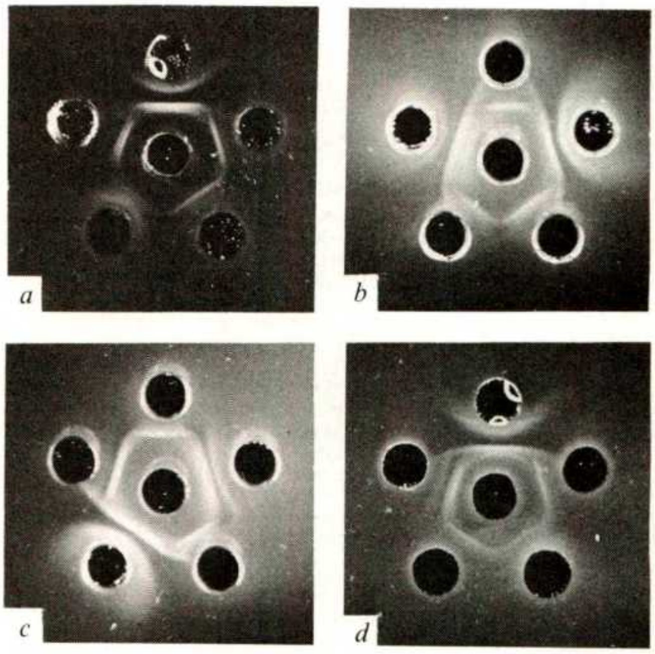

Fig. 1 Double diffusion precipitin reactions in agar gels. Immune serum (0.004 ml) is used in the centre wells and antigen extracts (35–40 µg cytosol protein), in the peripheral wells of micro-Ouchterlony plates (5-mm well spacings, Hyland Laboratories). The peripheral wells are designated as 1, at the top and sequentially numbered in a clockwise direction. In *a* the extracts are 1, total colonic mucosa; 2, adenocarcinoma of the colon (J.G.); 3, colonic surface (tip) cells; 4, colonic lower crypt cells and 5, placenta I. In *b* the extracts are 1, placenta I; 2, total colonic mucosa; 3, osteosarcoma, lung; 4, adenocarcinoma of the rectum (N.I.) and 5, ascites from metastatic adenocarcinoma of the colon (I.R.). In *c* the extracts are 1, adenocarcinoma of the colon (A.S.); 2, ovarian carcinoma; 3, adenocarcinoma of the rectum (C.W.); 4, total colonic mucosa and 5, colonic polyp. In *d* the extracts are 1, total colonic mucosa, 2, adenocarcinoma of the colon (I.R.); 3, colonic polyp; 4, adenocarcinoma of the colon (J.G.) and 5, placenta II.

Table 1 Purification of TK from human colonic mucosa

	Total protein (mg)	Total activity (µU)	Specific activity (µU per mg protein)	Yield (%)
Whole homogenate	9,940	52,682	5.3	
Supernatant (10^5g)	4,552	45,975	10.1	87.2
$(NH_4)_2SO_4$ precipitation (40%)	358	34,117	95.3	64.8
Heat at 50 °C (1 mM TdR)	143	28,629	200.2	54.3
$(NH_4)_2SO_4$ precipitation (40%), concentrated on Amicon after dialysis	64	25,446	397.6	48.3
Negative $Ca_3(PO_4)_2$ gel	22.7	19,034	838.5	36.1
Sephadex SG 200 (KCl) chromatography	6.9	10,554	1,529.6	20.0
BioGel P-150 chromatography	1.4	6,327	4,519.3	12.0
DEAE–cellulose chromatography	0.3	3,347	11,156.5	6.4

Pooled total mucosal scrapings (140 g frozen wet weight) from autopsy colons of patients with non-involvment of the gastrointestinal tract, obtained through the Tumour Procurement Service of Memorial Hospital, New York, were homogenised in a Waring Blendor at low speed with 0.25 M sucrose–0.2 M Tris-HCl (pH 8.2, 25 °C)–0.15 M KCl–0.17 M 2-mercaptoethanol (25:1.5:1:0.5), strained through gauze and centrifuged at 100,000g for 90 min. The pellet was washed and re-extracted twice and the combined supernatant fractions were adjusted to 40% saturation with respect to ammonium sulphate. The precipitate collected by centrifugation at 20,000g for 20 min was redissolved in 0.05 M Tris-HCl–0.25 M sucrose–6 mM 2-mercaptoethanol-1 mM TdR, heated at 50 °C for 5 min, rapidly cooled, centrifuged and reprecipitated with ammonium sulphate. The precipitate collected by centrifugation was dissolved and purified by absorption on calcium phosphate gel, followed by gel filtration in Sephadex SG-200 in the presence of salt and in BioGel 150 in the absence of salt and finally by elution with a linear salt gradient (0.05 M–1.0 M KCl) from DEAE–cellulose. This resulted in a 2,100-fold purification of the enzyme with respect to the activity in the whole homogenate. All steps were carried out at 4–8 °C. TK activity was assayed as before. A unit is defined as the enzyme phosphorylating 1 µmol TdR per min at 37 °C. For immunisation, 1 mg of purified colonic TK in 1.0 ml of 0.05 M Tris-1 mM TdR–6 mM 2-mercaptoethanol was emulsified by mixing with an equal volume of Freund's complete adjuvant and injected subcutaneously into New Zealand white albino male rabbits. This was followed a week later by a similar preparation with incomplete adjuvant. A booster injection with incomplete adjuvant was administered 6 weeks after the initial injection. Blood was collected from the marginal ear vein once a week for about 4 or 5 weeks after the booster.

Table 2 Residual activity of TK in various tissue extracts after preincubation with anti-human colonic TK from rabbits

Source of TK	Control TK activity* (pmol per incubation sample)	% Residual activity† Undiluted		
		Undiluted	1:10	1:1,000
Partially purified by a single precipitation with ammonium sulphate				
Normal colon	40±2	8	27	37
Adenocarcinoma colonic (J.G.)	66±3	54	67	86
Placenta I‡	68±2	60	77	87
Crude extract				
Ovarian carcinoma	80±4	35	48	65
PHA-stimulated lymphocytes	76±2	95	99	100
Myeloblastic leukaemic leukocytes	85±4	95	98	100

Cytosol TKs were prepared from surgical samples after homogenisation in the buffer described in Table 1, with a Potter–Elvejehm homogeniser and centrifugation at 127,000g for 60 min; the supernatant could be stored at –20 °C for more than 2 months with little loss in activity and at –80 °C for more than a year. Lymphocytes and leukaemic cells were washed with phosphate-buffered saline, pelleted, resuspended in a minimal volume of distilled water and lysed by repeated freezing and thawing. The lysed cell suspension was adjusted to the same concentration of homogenising medium and gently homogenised as were the surgical samples. TK activity was assayed as before[8].

*Figures are pmol per incubation sample. Control activity is the average of the enzyme activity in preparations preincubated with preimmune serum. The latter had no effect on the TK activity of any of the preparations tested. TK activity was assayed as before[8].

†Values signify percentage residual activity obtained with antisera relative to activity with preimmune serum. Various dilutions of both preimmune serum and serum (No. 317) of rabbit immunised with human colonic TK were preincubated overnight at 4 °C with equal volumes (0.20 ml containing 250–300 µg protein) of either dialysed preparations of TK partially purified by ammonium sulphate precipitation or crude 127,000g supernatant fractions. Three samples of the supernatant containing 50–60 µg antigen protein were removed and assayed for TK activity.

‡Placentae used in all these studies were flushed for at least 15 min with physiological saline through the umbilical artery. A portion of the foetal placenta free of maternal elements was removed and homogenised as described for surgical samples.

of the preparations examined. Antisera, which markedly inhibited homologous colonic TK were much less effective in neutralising the activity of enzymes from colonic adenocarcinoma, placenta (two samples, I and II) and ovarian carcinoma and did not inhibit the enzyme from either PHA-stimulated lymphocytes or myeloblastic leukaemic leukocytes (Table 2). The latter two preparations did not react with antisera to produce double diffusion precipitin lines in contrast to the extracts of total colonic mucoae, colonic crypt cells, colonic surface (tip) cells, placenta and tumours of diverse origin (Fig. 1). The precipitin lines were absent when supernatant from total colonic mucosa preincubated with undiluted antiserum was used as antigen, suggesting that these lines are essentially attributable to TK–antibody reactions. There were cross reactions between colonic TK and heterologous enzymes from placenta and colonic tumours (Fig. 1*a* and *d*), osteogenic sarcoma (lung) and placenta (Fig. 1*b*), rectal carcinoma and colonic polyp (Fig. 1*c*) and total mucosa from small intestines, suggesting partial antigenic identity of the enzymes in these preparations. There seemed to be cross reactions between TK from ascitic cells (metastatic colonic adenocarcinoma) and the enzymes from placenta and rectal adenocarcinoma (Fig. 1*b*). Precipitin patterns similar to those presented here for tumours were also observed in a breast carcinoma, a myxoliposarcoma, a gastric carcinoma and a cervical carcinoma.

In mammalian cells, there are multiple forms of TK[13-15], the relative proportions of which vary with the origin and the metabolic state of the tissue. The different precipitin patterns in the rapidly proliferating tissues examined could be a reflection of quantitative and/or qualitative differences in the isozymes present. The biphasic nature of the thermal inactivation observed in crude cytosol fractions[8] as well as in the partially purified colonic TK preparations suggests the presence of more than one form of the enzyme. Preliminary studies also indicate that there are at least three electrophoretic forms of TK in some of these tissues, and in the 2,100-fold purified TK used as antigen. In the latter, TK activity was associated with the three protein-staining bands which constituted about 90% of the total protein. There seems to be at least one form of TK in colonic tumours, polyps and placenta which is antigenically similar, but not identical, to that of normal colonic TK, most likely the form found in the surface cells.

Although TK activities from placenta and a colonic adenocarcinoma (J.G.) were inhibited to about the same extent (Table 2), the enzymes do not seem to be serologically identical (Fig. 1*d*). There is a similarity between these placental and tumour antigens but not the complete identity which has been reported for isozymes of human alkaline phosphatase[16]. TK with the electrophoretic migratory pattern characteristic of the enzyme found in the early stages of development of human foetal liver has been observed in malignant tumours from various sites[15,17,18]. Enzymes with similar migration, however, are not necessarily antigenically related, for example, from cervical carcinoma and the virus-specific enzyme induced by the epidemiologically associated herpesvirus type 2 (ref. 19). The similarity between TKs from placenta and tumours from various sources is reminiscent of the appearance of embryonal antigens in murine[20] and human[21] cancer cells.

The data presented here suggest that TK of colonic tumour is serologically more similar to the enzyme from surface cells than that from lower crypt cells. This would be consistent with the hypothesis that colonic tumours arise from surface epithelial cells rather than from the depths of the crypts[1,2].

We thank the members of the Department of Surgery for making surgical materials available. We also thank the Tumor Procurement Service for autopsy samples and other tumours. This investigation was supported by a Public Health Service grant from the National Cancer Institute through the Large Bowel Cancer Project and by a US Public Health Service grant.

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Received October 23; accepted December 30, 1975.

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Immunofluorescent localisation of human pregnancy-specific β-globulin in placenta and chorioepithelioma

VARIOUS immunological methods have been used to define the existence on plasma of several pregnancy-associated macromolecules[1–3], of which between three and five are now well recognised in humans[4,5]. The cells and tissues responsible for their synthesis, however, remain to be clearly defined, although, of the two pregnancy-associated β_1-globulins, one was recently detected in the sera of patients bearing trophoblastic tumours[6]. This particular β_1-globulin does not resemble any known hormone, including chorionic gonadotropin[7], but seems to be identical with the pregnancy-specific β_1-glycoprotein of Bohn[9] and the PAPP-C described by Lin *et al.*[5]. The placenta has been suggested as the site of its synthesis[8,9]. Using indirect immunofluorescence, we have now located this β_1-globulin in human placenta and chorioepithelioma of the uterus, where we believe it is synthesised.

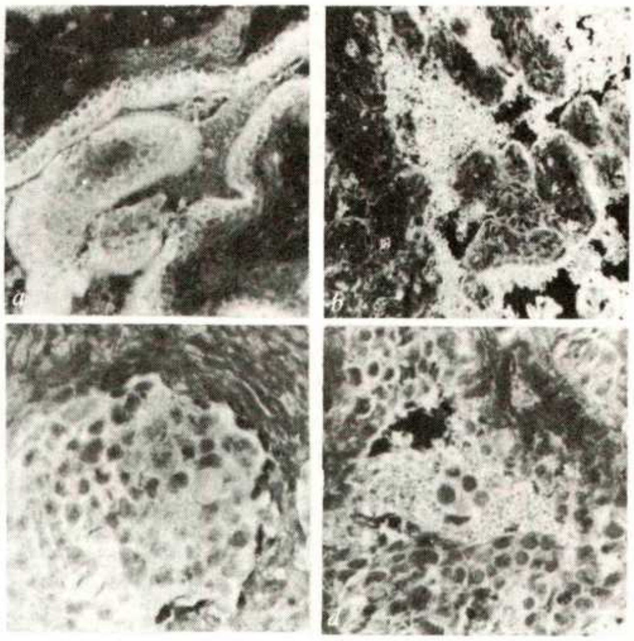

Fig. 1 *a*, Section of placental chorion (8-week gestation) treated with anti-β_1-globulin and anti-RGGFI, showing specific fluorescence in cytoplasm of cytotrophoblastic (Langhans) and syncytiotrophoblastic cells (×31). *b*, Section of the placenta (40-week gestation) treated with anti-β_1-globulin and anti-RGGFI, showing specific fluorescence in residual trophoblastic cells and in fibrin islands (×31). *c* and *d*, Sections of different chorioepitheliomas, showing specific fluorescence in cytoplasma and membranes of Langhans cells as well as in fibrin islands (also treated, ×52).

Samples of placenta were obtained from abortions, carried out at 6–12 weeks of gestation, or after normal birth. Chorioepitheliomas were obtained from affected patients at the time of hysterectomy. For controls, specimens of other organs were obtained at autopsy, 3–6 or 8–16 h after death, from patients with either chorioepitheliomas (without metastases) or non-malignant disease.

Tissue specimens (diameter 3–5 mm) were fixed in cold absolute ethanol with glacial acetic acid (99 : 1 v/v)[10] and subsequently embedded in paraffin and sectioned[11]. Anti-β_1-globulin was prepared as before[8]. Antibodies to anti-β_1-globulin and anti-albumin of human serum (anti-HSA) were raised in rabbits and then purified by the glutaraldehyde–immunosorbent technique[12]. Human albumin was chosen as the serum protein reference of nonspecific absorption because it is not produced by trophoblastic cells. The indirect immunofluorescence technique was used with donkey monospecific antibodies for rabbit γ-globulin labelled with fluorescent isothiocyanate (anti-RGGFI). Selected fields were photographed (ML-2B microscope) and subsequently stained with haematoxylin and eosin and mounted in glycerol buffer to facilitate comparison with definitively identifiable structures.

To ensure that the fluorescence exhibited by the anti-β_1-globulin sera was specific for the storage sites in the placenta and chorioepitheliomas, and to distinguish between specific and nonspecific uptake of the antibodies, the following controls were effected: (1) incubation of some sections only with anti-RGGFI; (2) use of anti-β_1-globulin neutralised with serum from pregnant women; (3) use of anti-HSA and anti-RGGFI on some sections, excluding the liver.

Specific immunofluorescence was found in placenta and chorioepitheliomas but not in lung, muscle, spleen, brain, pancreas, gastrointestinal tract or liver (when there was no metastasis or tumour). It was associated with the cytotrophoblast and syncytiotrophoblast of the chorion in immature placenta (Fig. 1*a*) and with residual trophoblast and fibrin islands, perhaps containing necrotic trophoblastic cells, in the term placenta (Fig. 1*b*). In chorioepithelioma, specific fluorescence was also associated with giant trophoblastic cells (Langhans cells) or fibrin islands (Fig. 1*c* and *d*).

The control experiments with 8-week gestation placental tissue and sections of liver after treatment with anti-HSA and anti-RGGFI showed little or no fluorescence.

We suggest that production of this particular β_1-globulin may start in the Langhans cells and continue in the syncytiotrophoblast of the normal chorion and in the trophoblast cells of chorioepitheliomas, where there may be less biosynthetic activity and secretory capacity.

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Immunological and biochemical characterisation of type C viruses isolated from cultured human AML cells

TYPE C viruses are associated in several species with naturally occurring malignancy and have been isolated from a variety of species including several non-human primates. The ability to specify interviral relationships is crucial for evaluating the degree of relatedness of new isolates to known viruses, especially those which are potentially associated with human malignancy. Recent studies of Gallagher and Gallo[1] indicated the presence of a type C virus in cultured cells from a patient with acute myelogenous leukaemia (AML) (patient A.S.). Viruses from this culture have been propagated in homologous and heterologous fibroblastic cells[2] in sufficient quantity to be characterised in detail.

Recently, immunological and molecular hybridisation methodology has been applied to the type-specific differentiation of known primate viruses[3-9]. The viruses that can be distinguished include three members of the related woolly monkey (SSV-1)-gibbon ape group and three members of the endogenous baboon virus-RD114 group (Table 1). In investigating the relationship of the AML virus isolate to known type C viruses using the above methodology, certain of our initial observations were previously recorded as "personal communication"[2]. It is our purpose here to amplify these findings. The data reported here were derived from virus produced by secondary fibroblastic cells[2] after infection with virus from primary leukocyte cultures of the first peripheral blood sample[1].

The AML virus (HL23V), propagated in either canine thymus or human cells was initially found to contain proteins of both primate virus groups based on complement-fixation tests and radioimmunoassays which are specific for woolly monkey–gibbon virus p30 and RD114–baboon virus p30, respectively. Growth of the HL23 virus in normal rat kidney (NRK) cells, which are relatively non-permissive for viruses of the RD114–baboon group, yielded a virus which only showed immunological reactivity characteristic of the woolly monkey–gibbon virus group.

The suggestion that two viruses were present in infected human and dog cultures was confirmed by detection of viral RNA using complementary ^{3}H-DNA (cDNA) prepared in the endogenous reverse transcriptase reaction. Uninfected cultures were negative in these assays. On the basis of the extent of its reaction using S1 nuclease, one of the viruses was indistinguishable from the woolly monkey virus (Fig. 1*a*). The woolly cDNA preparation hybridised to a lesser extent with the two gibbon (GALV) viruses and gave no hybridisation with cultures infected with the baboon or RD114 viruses or a culture replicating endogenous rat virus. The gibbon (SFMC) cDNA used gave the expected reciprocal reaction, namely a partial reaction with woolly monkey and AS virus-infected cell RNA (Fig. 1*b*). In view of the >95% reaction it was not surprising to find that thermal dissociation profiles of wooly cDNA, and woolly or HL23V RNA had midpoints (T_m) within 1 °C, whereas in the same conditions (hydroxyapatite chromatography) woolly monkey and gibbon heterologous DNA–RNA hybrid molecules showed a ΔT_m of 6.5 °C compared with homologous reactants (data not shown).

The second virus present has sequences indistinguishable from the endogenous baboon virus isolated from *Papio cynocephalus* (Fig. 1*c*). The cDNA preparation used distinguished this virus from a highly related endogenous virus isolated from the baboon species *Papio hamadryas*, which reached saturation at 80% of the homologous reaction and RD114: maximal reaction ~20%. The cDNA did not hybridise with human cell RNA including that of the cell line (uninfected) used to propagate the virus. No cross hybridisation was obtained with RNA from cells producing the woolly monkey virus. RD114 cDNA (Fig. 1*d*) gave the expected reciprocal reaction with baboon and AS viruses, namely ~20% at saturation. Note also that AS virus grown in rat cells did not hybridise with baboon or RD114 cDNAs, consistent with the immunological data indicating the presence of only the woolly monkey viral p30 in this culture. The relationship between the two baboon viruses seems closer than that of the woolly monkey–gibbon group based

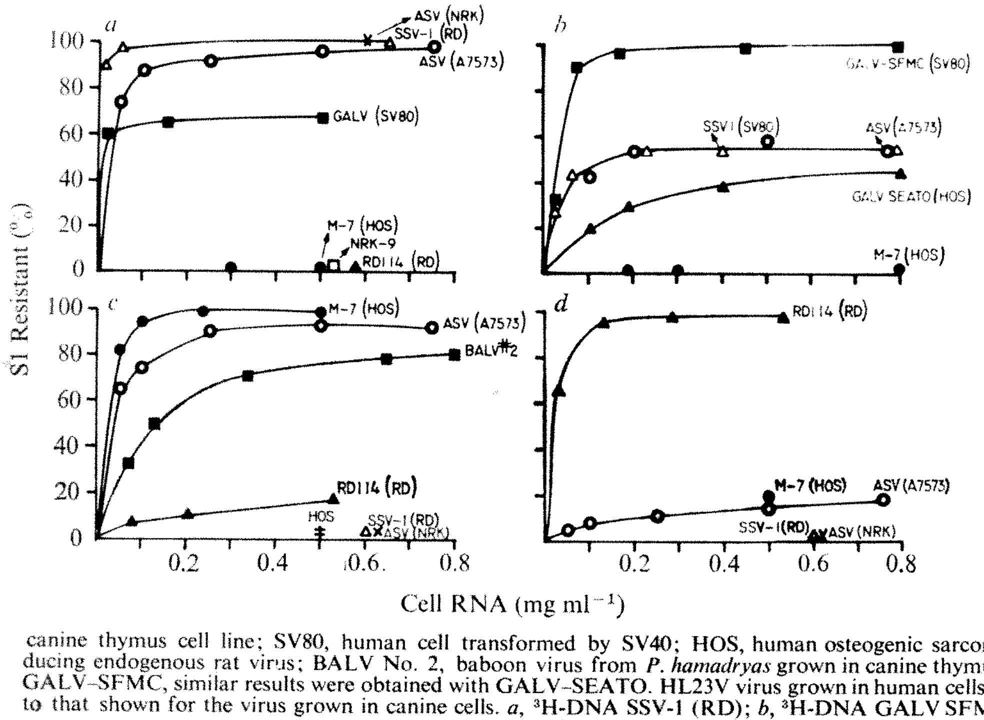

Fig. 1 ^{3}H-DNA transcripts (about 10^3 c.p.m. per assay) of woolly monkey virus (SSV-1) which represents the helper virus (SSAV) present in vast excess, the SFMC isolate of GALV, the baboon isolate of *Papio cynocephalus* (M-7), and the endogenous cat virus (RD114) were annealed with increasing amounts of cellular RNA. Incubation was at 67 °C for 18 h and mixtures were analysed by S1 nuclease digestion according to previously described methods[9]. The cDNAs used represent 60–70% of the various viral genomes based on resistance of labelled RNA to RNase digestion after annealing with excess cDNA. Values are normalised to 100% for the homologous reactants, actual values were 90% or greater for each of the four homologous sets of reactants. The cells in which the various viruses were grown are given in parentheses. NRK, Normal rate kidney; RD human rhabdomyosarcoma cell line; A7573, canine thymus cell line; SV80, human cell transformed by SV40; HOS, human osteogenic sarcoma cell line; NRK-9, rat cell producing endogenous rat virus; BALV No. 2, baboon virus from *P. hamadryas* grown in canine thymus cells. The data shown in *a* are for GALV–SFMC, similar results were obtained with GALV–SEATO. HL23V virus grown in human cells gave a similar hybridisation pattern to that shown for the virus grown in canine cells. *a*, ^{3}H-DNA SSV-1 (RD); *b*, ^{3}H-DNA GALV SFMC (SV80); *c*, ^{3}H-DNA M7 (HOS); *d*, ^{3}H–DNA RD114 (RD).

Table 1 Primate and related type C viruses distinguished by type-specific molecular hybridisation and immunoassays

	Virus	Origin
A (1)	Woolly monkey (SSAV-1)	Fibrosarcoma[10]
(2)	Gibbon ape (GALV-SFMC)	Lymphosarcoma[11]
(3)	Gibbon (GALV-SEATO)	Granulocytic leukaemia[12]
B (1)	RD114	Endogenous cat virus[13]
(2)	Baboon (BALV-M7-*Papio cynocephalus*)	Placenta[14]
(3)	Baboon (BALV No. 2-*Papio hamadryas*)	Lymph node of lymphomatous baboon[15]

Viruses of the *A* group are highly related as are those of the *B* group. The assays described in this report show no cross reactivity between groups. *B*-group viruses are endogenous in their species of isolation[16], while *A*-group viruses are not represented in cellular DNA of their species of isolation[17]. *A*-group viruses are believed to have originated in the remote past from ancestors of the genus *Mus*[18,19].

SFMC: San Francisco Medical Centre; SEATO: laboratory in Bangkok, Thailand.

on thermal dissociation data. Homologous heteroduplexes of the baboon viruses gave T_ms 2 °C higher than heterologous reactants. The HL23 virus, as expected from reaction extent, was indistinguishable by thermal stability criteria from the *Papio cynocephalus* virus (within 1 °C).

The distinct components of the HL23 virus preparation were further analysed in type-specific radioimmunoassays for low molecular weight viral structural polypeptides[3]. The HL23 virus and each of the woolly monkey–gibbon viruses showed complete cross reactivity in the woolly monkey virus p30 assay. In the woolly monkey p12 assay (Fig. 2*a*), HL23 virus and woolly monkey virus showed identical slopes and final reaction extents (complete displacement of the iodinated woolly monkey p12), while the two gibbon viruses were easily distinguished from the above viruses by these criteria. In the SFMC gibbon virus p12 assay, only the SFMC virus competed efficiently (Fig. 2*b*).

In analogous immunoassays for baboon–RD114 structural polypeptides (Fig. 3) both the AS virus and the endogenous virus of *P. cynocephalus* reacted efficiently in the *P. cynocephalus* virus p15 assay, but competed poorly in the RD114 p15 assay. In contrast, the *P. hamadryas* virus was poorly reactive in both p15 assays. RD114 competed completely in the homologous RD114 p15 assay but not in the *P. cynocephalus* p15 assay. In other studies the gp70s of the woolly monkey[4] and baboon components of the HL23 virus preparation were indistinguishable from the gp70s of the prototype woolly monkey virus and *P. cynocephalus* virus in competition assays for these polypeptides (data not shown).

To substantiate further the presence of two distinct viruses, HL23 virus preparations from canine thymus cells were mixed in separate tubes with neutralising antibody specific for baboon and woolly monkey virus and the mixtures added to human (RD) cells. At 3 weeks, cultures infected with HL23 virus plus anti-baboon serum contained only woolly monkey virus p30 and those infected with HL23 virus plus anti-woolly serum contained only baboon virus p30. It was also observed that passage of virus from diluted supernatant fluids readily selected for the woolly monkey virus component as might be expected from the severalfold excess of woolly virus proteins in the mixture.

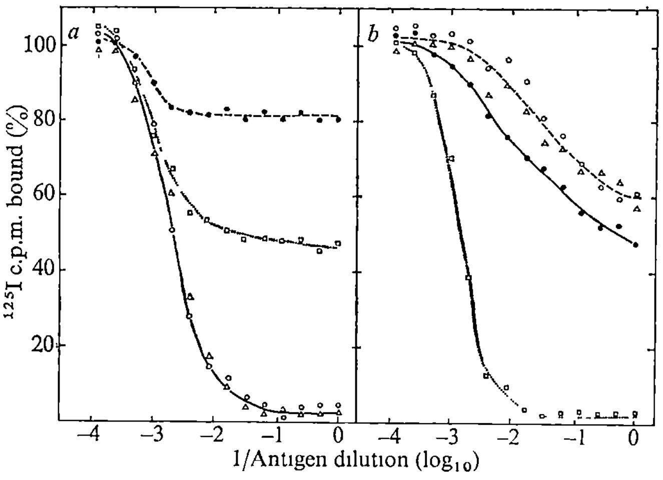

Fig. 2 Analysis of the HL23V virus in competition immunoassays for woolly monkey and gibbon ape virus p12 polypeptides[3]. Viruses were disrupted by incubation at 37 °C for 30 min in 1% Triton and were tested at serial twofold dilutions for ability to compete with ^{125}I-labelled p12 for binding limiting amounts of antiserum. Reaction mixtures contained 0.01 M Tris, 0.1 M NaCl, 0.15 M EDTA, 0.1% Triton X-100, and 1% bovine serum albumin (*p*H 7.8) in a volume of 0.7 ml Antiserum and unlabelled competing antigen were incubated at 37 °C for 1 h; ^{125}I-labelled antigen was added, and the incubation continued for 3 h at 37 °C and a further 18 h at 4° C. After addition of 0.025 ml undiluted swine anti-goat IgG to each tube, the mixture was incubated for 3 h at 4° C, centrifuged at 2,500 r.p.m. for 15 min and the resulting precipitate measured for radioactivity. Viruses tested included the woolly monkey virus[10] (○), SFMC gibbon ape virus[11] (□), SEATO gibbon ape virus[12] (●), and the HL23V virus[1,2] (△). *a*, Competition immunoassay using antiserum against woolly monkey virus to precipitate ^{125}I-labelled woolly monkey virus p12; *b*, competition immunoassay using antiserum against SFMC gibbon ape virus to precipitate gibbon ape virus p12.

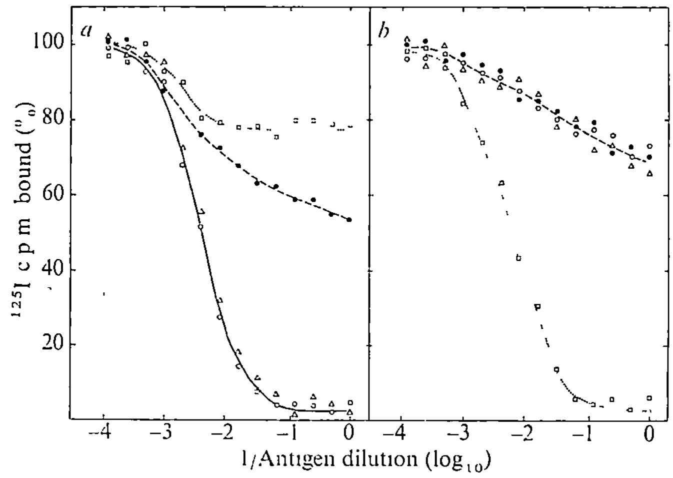

Fig. 3 Analysis of HL23V virus in competition immunoassays for the baboon virus and RD114 p15 (ref 5). Viruses were assayed as described in the legend to Fig. 2 and included the *P cynocephalus*[14] (○) and *P. hamadryas*[15] (●) baboon viruses; RD114[13] (□); and the AS virus[1,2] (△) *a*, Competition immunoassay using an antiserum against *P. cynocephalus* baboon virus to precipitate ^{125}I-labelled *P. cynocephalus* baboon virus p15; *b*, competition immunoassay using an antiserum against RD114 to precipitate ^{125}I-labelled RD114 p15.

The present studies thus show that HL23 virus preparations contain two distinct viruses; one indistinguishable from the woolly monkey virus, the second indistinguishable from an endogenous virus of the baboon species, *Papio cynocephalus*.

These data pose critical questions concerning the origin of the HL23 viruses. The high degree of relatedness to two known viruses constitutes argument for accidental contamination, yet other data (refs 1, 2 and refs quoted therein) have suggested the possibility of direct origin of the viruses from fresh cells of the patient. The important question is that of the relationship of these viruses to human disease, an issue which is under active investigation in several laboratories.

This study was supported by the US National Cancer Institute and the US Public Health Service.

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Characterisation of a virus (HL23V) isolated from cultured acute myelogenous leukaemic cells

THE isolation of a type C RNA tumour virus (HL23V) from cultured human acute myelogenous leukaemia peripheral blood leukocytes has recently been reported[1,2]. The reverse transcriptase of this putative human oncornavirus was found[1] to be immunologically related to the reverse transcriptase of the simian sarcoma virus, type 1 (SSV-1), the gibbon ape lymphoma virus (GALV), and to a small extent in secondary culture, RD114 (ref. 2). The spontaneously released virus was shown to be infectious for several cell cultures, including a human rhabdomyosarcoma cell line, A204 (ref. 2). This culture produced virus in sufficient quantities to permit biochemical studies. Here we describe the characterisation of the virus(es) grown in this culture. We find that the particles produced by the A204 culture infected with HL23V are a mixture of two viruses that are indistinguishable immunologically and in nucleotide sequence from two known non-human primate viruses, the baboon endogenous virus (BV-M7)[3,4] and the woolly monkey virus (SSV-1)[5].

Our findings complement those reported by Okabe *et al.*[6] elsewhere in this issue. These investigators looked for similarities with known oncornaviruses by molecular hybridisation with the cytoplasmic RNA from various cell lines infected with HL23V and by antigenic analysis using the type-specific p12 and p15 antigens. Our investigation emphasised molecular hybridisations with the RNA found in the particles released into the supernatant by A204(HL23V) and our antigenic analysis focused on the major structural antigens (p30 and gp45 of SSV and the p28 of BV-M7).

The A204(HL23V) culture produced high titres of particles that were found by ^{3}H-uridine labelling to possess a characteristic oncornavirus buoyant density of 1.16 g ml^{-1}. Simultaneous detection[7] assay of the culture supernatants demonstrated that the particles encapsulated 70S RNA and reverse transcriptase. The ^{3}H-DNA synthesised in simultaneous detection reactions and sedimenting as 70S complementary DNA (cDNA)–RNA complexes served as the source of the cDNA probe used in all the hybridisation experiments described here.

The reverse transcriptase from A204(HL23V) culture supernatants was examined for relatedness to the SSV enzyme. In contrast to the report of Gallagher and Gallo[1] (which indicated greater relatedness) but in agreement with Teich *et al.*[2], the results demonstrated (data not shown) that antisera prepared against the SSV reverse transcriptase was capable of inactivating the reverse transcriptase activity of HL23V particles only to about 60%. This partial inhibition of enzyme activity suggested the presence of a second virus containing an antigenically unrelated enzyme. To identify the unknown component in the HL23V particles, a search was instituted among known animal oncornaviruses using both immunological and molecular hybridisation techniques. The possibilities were quickly narrowed to the RD114/CCC baboon endogenous virus group.

Figure 1*a* and *b* shows hydroxyapatite temperature gradient elution profiles of viral cDNAs prepared from SSV and BV-M7 annealed to total RNA from HL23V particles. The extent of hybridisation and the thermal stability indicate that the HL23V particles contain the complete information of both the simian sarcoma virus (SSV) and the baboon endogenous virus isolated from *Papio cynocephalus* (BV-M7).

To determine whether all of the genetic information of HL23V can be accounted for by these two viruses, the reciprocal hybridisations were performed. In these experi-

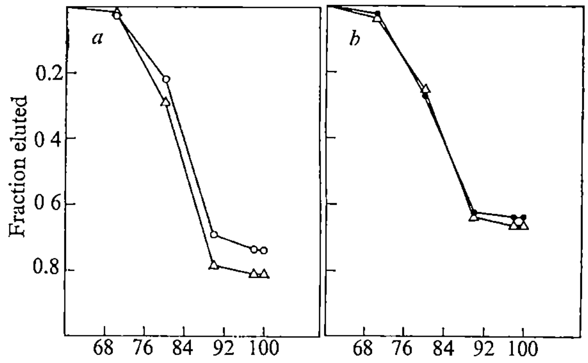


Fig 1 Hybridisation of ^{3}H-DNA transcripts of SSV-1 (*a*) and BV (M7 isolate) (*b*) to HL23V viral RNA. SSV-1 and BV were obtained from Pfizer, Inc. and were derived from sucrose density gradient-banded tissue-culture supernatants of a chronically infected human lymphoblastoid cell line (NC-37) and baboon kidney–canine thymus coculture (BKCT), respectively. HL23V virus was prepared from high speed pellets of 2-d-old media from HL23V-infected human rhabdomyosarcoma cell cultures ^{3}H-DNA probes for each of the viruses were isolated from the 60–70S RNA–DNA hybrids of standard large scale simultaneous detection assays. Hybridisation reactions were set up between the various probes (500–1,000 c.p.m. per assay) and viral RNAs (0.2–1.0 μg) in 20-μl volumes in sealed siliconised glass tubes in 0.8 M phosphate buffer (pH 6 8) 0.1% SDS and 10 mM EDTA. After heating at 100 °C for 1 min, the reactions were incubated at 68 °C for 20 h ($C_{r0}t > 2$). The reactions were analysed by thermal elution hydroxyapatite chromatography. Fractions of the total radioactivity eluted above 60 °C were plotted as a function of temperature. ○, SSV-1 RNA; ●, BV RNA; △, HL23V RNA.

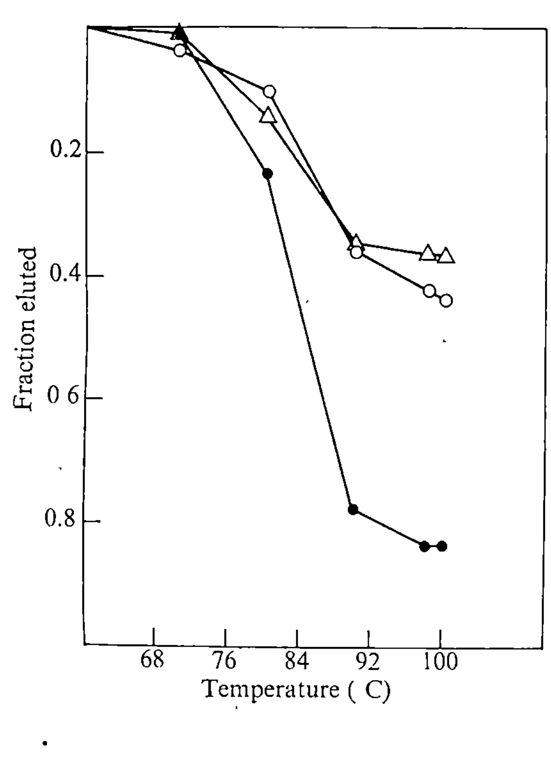
Table 1 Analysis of HL-23 for SSV genomic content by competition hybridisation

Competing RNA	Competing RNA ^{125}I-SSV RNA	Resistance of ^{125}I-SSV RNA %
None	—	72
SSV-1	1,500	3
GALV	1,200	15
MuLV-R	1,800	75
Mouse 18S+20S	450	74
HL-23	2,300	6

Hybridisation reactions (5.5 μl) were performed as described in Fig. 2 and contained 0 11 ng ^{125}I-SSV RNA (1.2×10^8 c.p.m. μg^{-1}), 0.68 ng ^{3}H-SSV cDNA (2×10^3 c p.m. μg^{-1}) and 0.5–0.25 μg of the indicated competing RNA. Following incubation at 68 °C for 48 h, the reactions were diluted with 0.01 M Tris-HCl (*p*H 8.0) 0.4 M NaCl, 0.01 M EDTA and divided into four equal aliquots. RNase A (25 U ml^{-1}) and RNase T$_1$ (5 U ml^{-1}) were added to two aliquots and the samples incubated at 37 °C for 1 h. Nuclease resistance was the ratio of acid-precipitable ^{125}I in the samples with and without ribonuclease. Recovery of input acid-precipitable ^{125}I was greater than 90%. SSV RNA was iodinated by the procedure of Commerford[8] as modified by Tereba and McCarthy[9]. SSV RNA was isolated from virions by disruption with SDS, Pronase treatment, rate sedimentation in a sucrose–SDS gradient and equilibrium density gradient centrifugation in potassium iodide. Viral RNAs from GaLV and MuLV-R were isolated by similar procedures excepting the KI gradient. HL23 VRNA was total RNA from purified virus and the mouse 18S and 20S RNAs were extracted from purified ribosomal subunits from NIH/3T3 tissue culture cells. ^{3}H-SSV cDNA was synthesised from SSV RNA and oligo(dT) with AMV DNA polymerase in the presence of 0.1 mg μl^{-1} actinomycin D and 0.5 mg ml^{-1} distamycin A. The reaction contained TTP, dATP and dGTP at 1 mM each and ^{3}H-dCTP (25 Ci mmol^{-1}) at 0.05 mM This SSV cDNA protected ^{125}I-SSV RNA 33%, 75% and 87% from ribonuclease digestion at molar input ratios of 0.4, 3 and 15, respectively (cDNA–RNA).

ments, cDNA probe was synthesised with HL23V particles and was annealed to RNAs from SSV-1 or BV-M7 or both. Figure 2 shows that the HL23V cDNA hybridised 37% and 44% to the BV-M7 and SSV-1, respectively. These individual hybridisations were additive as demonstrated by the complete complexing of HL23V cDNA to a mixture of BV-M7 and SSV-1 viral RNAs. These data indicate that the genetic information of HL23V virions is accounted for, within the limits of the sensitivity of the molecular hybridisation techniques used. It would seem that the HL23V genetic information contains the genomes of both SSV-1 and the BV-M7.

Fig. 2 Reconstruction hybridisations HL-23 ^{3}H-DNA probe was hybridised to SSV and BV RNAs individually and in combination. ○, SSV RNA; △, BV RNA; ●, SSV and BV RNAs.



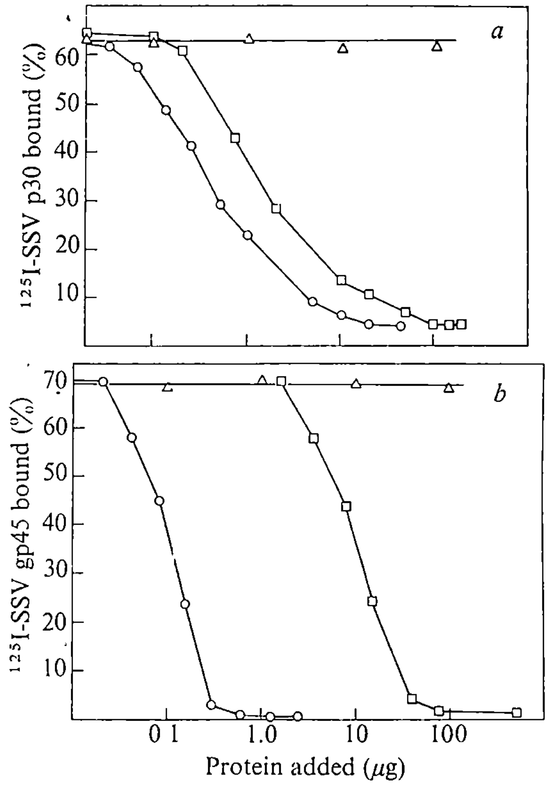


Fig. 3 Competition radioimmunoassays. HL23V virus was assayed for the presence of proteins antigenically related to the p30 (*a*) and gp45 (*b*) of NC-37 grown SSV-1. Antisera used for the studies with SSV-p30 were prepared by immunising rabbits with the purified p30. An NC-37 absorbed rabbit antiserum prepared by inoculation of disrupted virus and used for the studies with SSV-gp45 was kindly supplied by Dr D. Larson (Pfizer, Maywood, New Jersey). The p30 protein of SSV-1 was purified from sonicated virus, followed by phosphocellulose and Sephadex G75 column chromatography and isoelectric focusing It was iodinated and repurified as described by Hunter[18]. The iodinated p30 antigen preparations obtained had specific activities of 6×10^6 c.p.m. μg^{-1}. The gp45 protein of NC-37 grown SSV was purified from sonic-disrupted virus by column chromatography on Agarose 5M guanidium hydrochloride Analysis of the preparations by 5% sodium dodecyl sulphate polyacrylamide gel electrophoresis and isoelectric focusing indicated homogeneity of the 45,000-dalton antigen. The viral protein was labelled by the procedure of Bolton and Hunter[19]. Succinimidyl-(3-4-hydroxphenyl) proportionate (ICN Pharmaceuticals, Inc.) was first labelled with ^{125}I and purified. The purified gp45 was then labelled by conjugation with the ^{125}I-labelled ester and chromatographed on a Sephadex G-25 column. Titrations of the antisera were performed in 200-μl reactions that included 0.2% bovine serum albumin (BSA) in saline, 5,000 c.p.m. of ^{125}I-antigen and dilutions of antibody. After 1 h incubation at 37 °C, 100 μg of normal rabbit carrier IgG and a titred amount of goat anti-rabbit IgG were added. The reaction was incubated for 15 h at 4 °C. The samples were centrifuged and both precipitates and supernatants were counted in a Searle Autogamma counter model 1185. The results are expressed as percentage c.p.m. precipitated. More than 80% of the labelled antigen could be bound by specific antisera. Competition assays were performed in a similar manner, except that unlabelled competing antigen was added to the original incubation mixture. The unlabelled competing antigens were SSV (○); HL-23 (□); SSV-gp45 (●) and MPMV (△)

To supplement and confirm these findings by an independent method, competition molecular hybridisations were performed using cDNA synthesised from SSV. Viral 70S

RNA from SSV was radiolabelled with ^{125}I. SSV cDNA can protect this ^{125}I-SSV RNA more than 79% from ribonuclease digestion at molar input ratios of 5 : 1 (cDNA–RNA). Table 1 shows that when cold viral and other RNAs were added in vast excess to complete this homologous reaction, both SSV and HL23V RNA rendered the ^{125}I-SSV completely digestible. The GALV-1 competed less successfully, illustrating the sensitivity of this technique for detecting the small sequence differences known to exist between GALV and SSV.

HL23V was further tested for its immunological similarity to SSV and BV-M7. Virus was concentrated by ultracentrifugation from culture supernatants of A204(HL23V) and used as competing antigens in radioimmunoassays for the p30 and gp45 of NC-37 grown SSV. As shown in Fig. 3, the slope and extent of competition of HL23V in both of these radioimmunoassays were indistinguishable from SSV. Immunodiffusion analysis of HL23V with antisera prepared against the major internal structural proteins of the woolly monkey (p30) and baboon viruses (p28) demonstrated lines of identity with BV-M7 and SSV-1 indicating that, by these immunological criteria, HL23V is indistinguishable from a mixture of these two agents.

The association of SSV or BV with human material is not confined to the one patient from which HL23V was presumably derived. Four groups have now reported the isolation of agents related to SSV[1,10–12] from a variety of normal and neoplastic human tissues Further, in 7 out of 40 cases of acute myelogenous leukaemia, where a decision could be made, an enzyme was found[13,14] that was neutralised by anti-SSV reverse transcriptase. Fresh[15] or short term cultures[16] of human leukaemic cells have been shown to contain particles capable of generating cDNA having significant homology to SSV. Sherr and Todaro[17] have reported that two human tumours of 33 examined by radioimmunoassay demonstrated BV-M7 p28. The repeated encounters with SSV or BV-related material in human samples suggest that these viruses may be more widespread in the human population than had been suspected.

The fact that the HL23V particles have been identified as known animal viruses does not of itself eliminate them as candidate aetiological agents of the human disease. At the same time, it must be accepted that the identification of unknown tissue culture viruses with known laboratory agents raises the obvious possibility that they arose by contamination.

The key question is of course the relationship of the HL23V particles to human leukaemia. A resolution of this issue with respect to any candidate human tumour virus growing in culture requires answers to the following two questions: (1) Can one provide evidence at the level of protein and/or nucleotide sequence for the presence of the putative agent in the original tumour material from which the tissue culture was established? (2) Can one provide evidence at the level of protein and/or nucleotide sequence for the presence of the putative agent in the tumour cells of other patients with the same disease? Finding evidence for the particles in the original tumour cells would serve to eliminate the trivial explanation that they arose in the culture through laboratory contamination. The answer to the second question will decide the general relevance of the observation to human leukaemia. Unless a positive response is obtained in a large proportion of the patients examined, no basis exists for identifying the HL23V particles as clinically significant aetiological agents of human leukaemia.

We thank Drs J. Gruber and D. Howell for viral and immunological reagents, Dr R. Wilsnak for anti-SSV reverse transcriptase and anti-BV p28, and T. Mederios, B. Hills, B. Ma, P. Moy, I. Doneva and K. Tirk for technical assistance.

This study was supported by grants from the US Public Health Service and US National Cancer Institute.

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Received November 26, 1975; accepted February 3, 1976.

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Construction of a P plasmid carrying nitrogen fixation genes from *Klebsiella pneumoniae*

THE wide host range of plasmids of the P-incompatibility class[1,2] has attracted interest in this group of R factors. A 3×10^6-dalton sequence which includes the TEM β-lactamase gene found on some plasmids of this group can integrate into the *Escherichia coli* chromosome[3] and into plasmids of other compatibility groups[1,4,5]. This translocatable sequence has been termed transposon A (TnA)[4,6] and resembles the insertion sequences (IS) of *E. coli*, in that TnA insertions can result in gene inactivation, giving rise to strong polar mutations[7].

We have described[8] the construction of an F-prime factor, FN68, carrying the nitrogen fixation (*nif*) and *his* region of the *Klebsiella pneumoniae* chromosome; other chromosomal markers detected on this plasmid include *met*G, *gnd* and *shi*A, though it is uncertain whether these determinants originated from *Klebsiella* or from *E. coli*. FN68 carries a TnA sequence which was translocated from R68, a plasmid of the P-incompatibility group. We have observed that FN68 can interact with the P plasmid RP4, which also possesses the TnA sequence, resulting in the formation of a recombinant molecule which retains P-incompatibility properties and carries the *nif his* region of the *K. pneumoniae* chromosome.

The P-group R factor RP4, which determines resistance to kanamycin (Km^R), tetracycline (Tc^R) and carbenicillin (Carb^R) was introduced into an *E. coli* K12 strain SB1801, carrying FN68. The resultant strain SB1801 (RP4) (FN68) was mated in broth with a His$^-$ recipient *E. coli* UNF502 (Table 1, Experiment 1). F-prime transfer (selection for *his*) occurred at a higher frequency than R-factor transfer (selection for Km^R) but cotransfer of both markers was not infrequent. Ten of the

Table 1 Transfer properties of *nif* plasmids in *E. coli* K12 strains

Exp. no.	Donor	Recipient	Selection	Mating	Transfer frequency	Exconjugant pheontype
1	SB1801 (FN68) (RP4)	UNF502	His$^+$	B	3×10^{-2}	
			Km^R	B	7×10^{-5}	
			His$^+$Km^R	B	3×10^{-6}	10/20 His$^+$Nif$^+$Carb^{R}Tc^{R}Km^{R}MS2^{R}PRR1^S
2	JC5466 (RP41)	AB2463	Km^R	S	5×10^{-1}	29/29 Carb^{R}Tc^{R}His$^+$Nif$^+$
			His$^+$	S	5×10^{-1}	28/29 Km^{R}Carb^{R}Tc^{R}Nif$^+$ 1/29 Km^{R}Carb^{S}Tc^{R}Nif$^+$
3	JC5466 (RP41)	SB1801	Km^R	S	5×10^{-1}	10/10 Carb^{R}Tc^{R}His$^+$Nif$^+$
			His$^+$	S	5×10^{-1}	10/10 Carb^{R}Tc^{R}Km^{R}Nif$^+$
4	JC5466 (RP41)	SB1801	Km^R	B	2×10^{-3}	10/10 Carb^{R}Tc^{R}His$^+$Nif$^+$
			His$^+$	B	2×10^{-3}	10/10 Carb^{R}Tc^{R}Km^{R}Nif$^+$

Donors in broth matings (B) were late log-phase cultures grown in nutrient broth with slow shaking at 37 °C and contained about 2×10^8 bacteria per ml. Recipients were grown overnight in nutrient broth without shaking. For agar surface matings (S), 0.1 ml of a late-log donor culture was mixed with 0.1 ml of a late-log recipient culture and the mixture plated on nutrient agar for 6 h at 37 °C. Bacteria were scraped off the surface of the plates, resuspended in saline phosphate buffer and plated on selective media. Transfer frequencies were calculated relative to the number of donors in the mating mixture at the time of plating on selective media. Transfer frequencies determined for surface matings can only be approximate since differential growth of donor and recipient occurred during the prolonged incubation of mating mixtures on agar medium. *E. coli* K12 strains were:

SB1801, *his*-750 (deletion covering *his gnd* RHA-2A) *str*^{R} λ^R *ara*$^-$ *gal*$^-$ *mal*A$^-$ *xyl*$^-$ m*tl*$^-$ (λ$^-$);
AB2463, *thi*$^-$ *arg*$^-$ *his*-4 *pro*$^-$ *thr*$^-$ *leu*$^-$ *str*^{R} *rec*A13 *lac*$^-$ *gal*$^-$ *ara*$^-$ *xyl*$^-$ *mtl*$^-$;
UNF502, *thi*$^-$ *arg*$^-$ *his*-4 *pro*$^-$ *thr*$^-$ *leu*$^-$ *str*^{R} *lac*$^-$ *gal*$^-$ *ara*$^-$ *xyl*$^-$ *mtl*$^-$ *nal*^{R};
JC5466, *trp*$^-$ *rec*A56 *his*$^-$ *spc*^{R}.

Plasmids were:
FN68, F-incompatibility group F′ *met*G$^+$ *gnd*$^+$ *his*$^+$ *nif*$^+$ *shi*A$^+$;
RP4, P-incompatibility group Km^R Carb^R Tc^R;
RP41, recombinant plasmid described in the text.

Km^R, Resistance to kanamycin sulphate, 30 μg ml^{-1}, Tc^R, resistance to tetracycline-HCl, 15 μg ml^{-1}; Carb^R, resistance to carbenicillin (Beechams), 200 μg ml^{-1}.

twenty His$^+$ Km^R exconjugants obtained were Carb^R Tc^R Nif$^+$ and sensitive to P-specific phage PRR1, but had lost sensitivity to F-specific phage MS2. Several of these clones cotransferred *his nif* and Km^R to the *E. coli rec*A strain JC5466. One derivative of JC5466 was studied in detail; it contained a plasmid designated RP41 which cotransferred Km^{R}Carb^{R}Tc^R *his* and *nif* at high frequency in matings carried out on an agar surface (Table 1, Experiments 2 and 3) but at low frequency in broth matings (Table 1, Experiment 4). Such differences between surface and liquid matings are characteristic of P plasmids[8].

RP41 did not coexist with the P-group plasmid, R751; when selection was made for trimethoprim resistance (a marker on R751), 35 exconjugants tested were His$^-$ Nif$^-$ and sensitive to tetracycline, kanamycin and carbenicillin (Table 2, Experiment 3). RP41 and the F-prime factor F′Cm, however, coexisted stably (Table 2, Experiments 4 and 5). These results suggest that RP41 has P-group incompatibility and does not retain the incompatibility properties of F. RP41 was stable in the *E. coli rec*A host JC5466 and no loss of *his nif* or drug resistance was detected after 15 generations in nutrient broth.

The nitrogenase activity of JC5466 (RP41) as measured by acetylene reduction *in vivo*[8] was 60 mol ethylene per mg protein per min, compared with 63 mol ethylene per mg protein per min for *K. pneumoniae* strain HF3 which has chromosomally located *nif* genes; both strains having been assayed in identical conditions. The host strain JC5466 did not reduce acetylene. In addition to *his* and *nif* genes, RP41 also probably carries a determinant for gluconate-6-phosphate dehydrogenase (*gnd*) since a *gnd* deletion strain *E. coli* SB1801 gave a positive reaction in the colorimetric assay for this enzyme[9] when RP41 was present. When RP41 was transferred to AB 2880, a His$^-$ShiA$^-$AroD$^-$ recipient, His$^+$ exconjugants inherited *shi*A, a marker located close to *his*, but not *aro*D. In contrast to the F′*nif*, FN68, RP41 does not carry *met*G, since cotransfer of this determinant with *his* was not detected in a cross with a His$^-$ MetG$^-$ derivative of *E. coli* C, and 30 His$^+$ exconjugants derived from this cross did not grow in the absence of methionine. RP41 thus carries the determinants *gnd his nif shi*A derived from FN68, but lacks *met*G.

Covalently closed-circular (CCC) DNA isolated from *E. coli* strain J62-1 carrying RP41 sedimented at 72S in neutral sucrose and 171S in alkaline sucrose gradients (Fig. 1) corresponding to a molecular weight of 59×10^6.

RP41 was transferable from *E. coli* to *K. pneumoniae* at high frequency. When the recipient was *K. pneumoniae* Kp52, a *nif his* deletion strain, no segregation of plasmid markers was detectable (Table 3, Experiment 1). Drug resistance, *his* and *nif* were cotransferred from *K. pneumoniae* Kp52 (RP41) to *E. coli* at high frequency (Table 3, Experiment 2).

It was also possible to transfer RP41 to a His$^-$ derivative of the unrelated bacterium *Agrobacterium tumefaciens*; cotransfer of *his* and drug resistance was observed but 10 clones tested were Nif$^-$ and did not give acetylene reduction in either anaerobic or aerobic conditions (Table 3, Experiment 3). Crude extracts of one of the His$^+$ exconjugants grown with low aeration in nitrogen-free medium containing aspartate (100 μg ml^{-1}), however, produced material which cross reacted antigenically (CRM) with antiserum prepared against purified *K. pneumoniae* nitrogenase molybdoprotein. No CRM was detected in extracts prepared from cultures grown on NH_4^+

Table 2 Compatibility properties of RP41

Exp. no.	Donor	Recipient	Selection	Transfer frequency	Exconjugant phenotype
1	J62-2 (R751)*	SB1801	Trim	>1	
2	J62-2 (R751)*	SB1801 (RP4)	Trim	2×10^{-4}	
3	J62-2 (R751)*	SB1801 (RP41)	Trim	1×10^{-4}	35/35 Trim^R Carb^S Tc^S Km^S His$^-$ Nif$^-$
4	F′Cm†	SB1801	Cm	7×10^{-1}	
5	F′Cm†	SB1801 (RP41)	Cm	2×10^{-1}	36/36 Cm^R Carb^R His$^+$ Nif$^+$ Tc^R Km^R

Matings were carried out on an agar surface* or in broth† as described in the legend to Table 1. *E. coli* K12 strain: J62-2 *pro*$^-$ *his*$^-$ *trp*$^-$ *lac*$^-$ *spc*^{R}. Plasmids: F Cm, F prime factor, determines chloramphenicol resistance, (Cm^R) 30 μg ml^{-1}, R751, P-group plasmid, determines resistance to trimethoprim lactate (Trim), 50 μg ml^{-1}. All other bacterial strains, plasmids and symbols are as in Table 1.

as 1 mg $(NH_4)_2SO_4$ per ml, a nitrogen source which represses nitrogenase synthesis, in *Klebsiella*. When RP41 was transferred back to *E. coli*, several exconjugants were Nif$^+$, confirming that *K. pneumoniae nif* genes had been transferred to *Agrobacterium* (Table 3, Experiment 4).

Transfer of RP41 to *Rhizobium meliloti* occurred at low frequency and some segregation of plasmid markers was observed; only 2 out of 10 His$^+$ exconjugants were tetracycline resistant and only 12 of 20 Tc^R exconjugants were His$^+$ (Table 3, Experiment 5). RP41 was unstable in *R. meliloti;* a 48% segregation of *his* was observed after 3 subcultures in L broth. Ten His$^+$ and twenty Tc^R exconjugants did not reduce acetylene when tested in either aerobic or anaerobic conditions. A faint band of cross-reacting material for *Klebsiella* nitrogenase molybdoprotein was observed in extracts prepared from cultures grown on nitrogen-free medium containing aspartate but not from NH_4^+-repressed cultures. *Nif* genes were expressed when the plasmid was transferred back to *E. coli*, indicating that *K. pneumoniae nif* genes were present in *R. meliloti* (Table 3, Experiment 6).

Transfer of RP41 to *Pseudomonas aeruginosa* was not observed. Two *his*$^-$ strains known to accept RP4 (strains PA067 and PAT904) were tested by surface and liquid matings and no His$^+$ or Carb^R derivatives appeared.

Although the precise mechanism of the recombinational events described here remains obscure, it is probable that cointegration was promoted by insertion sequences associated with the β-lactamase genes present in both parent plasmids. We suggest that cointegration of FN68 and RP4 resulted in excision of a segment of FN68 DNA which included F sex factor genes and *met*G, by a process analogous to that shown for other plasmids formed *in vivo* by site-specific recombination[10]. RP41 may resemble a similar plasmid, RP1-*his* described by Olsen and Gonzalez[11]. Both plasmids show stable linkage of drug-resistance genes and *his* in *E. coli* but both plasmids dissociate in intergeneric matings.

A P plasmid carrying nitrogen fixation genes has many advantages over an F'*nif* factor for genetic studies of nitrogen fixation. It transfers at high frequency from *E. coli* to *K. pneumoniae* and retains transferrability in this host in contrast to the F'*nif* FN68 which is Tra$^-$ in *Klebsiella*[8]. Mutants of RP41 could therefore be useful for complementation analysis of the *K. pneumoniae nif* region. RP41 also has a wide host range and should be transmissible to many Gram-negative bacteria, although the failure to obtain transfer to *Pseudomonas aeruginosa* suggests that the host range has been modified.

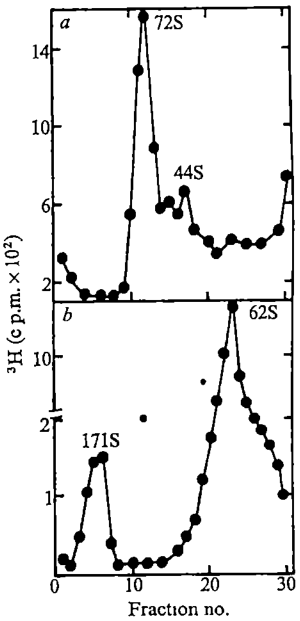

Fig. 1 Profiles of ^{3}H-labelled DNA from *E. coli* J62-1 (RP41) analysed by velocity centrifugation in neutral (*a*) and alkaline (*b*) 5 ml, 5–20% sucrose gradients. Supercoiled DNA, obtained from ethidium bromide–CsCl gradients[8], was dialysed against TES buffer (10 mM Tris-HCl, 1 mM Na_2EDTA, 100 mM NaCl *p*H 8.0). RP41 DNA (180 μl) was mixed with 20 μl of ^{14}C-labelled ColE1 DNA, layered on gradients and centrifuged for 60 min (*a*) and 33 min (*b*) at 120,000*g* at 20 °C in a 52 rotor using a Christ ωII ultracentrifuge. Gradients were fractionated by drop collection (7 drops per fraction) directly on to filter disks and TCA-precipitable label assayed for radioactivity.

The failure to express *K. pneumoniae nif* genes in *R. meliloti* is perhaps not surprising, since *R. meliloti* itself has *nif* genes which were not expressed in the conditions tested, but which are expressed in legume root nodules. It will be interesting to observe whether the *K. pneumoniae nif* system can be expressed in *R. meliloti* in symbiotic conditions or in the conditions developed for expression of nitrogenase in certain free-living rhizobia[12–16]. We did find CRM to antiserum to *Klebsiella* nitrogenase molybdoprotein in extracts of derepressed cultures

Table 3 Intergeneric matings with RP41

Exp. no.	Donor	Recipient	Selection	Transfer frequency	Exconjugant phenotype
1	*E. coli* K12 JC5466 (RP41)	*K. pneumoniae* M5a1 Kp52	His$^+$ Km^R	1×10^{-1} 1×10^{-1}	87/87 Km^{R}Tc^{R}Carb^{R}Nif$^+$ 56/56 Tc^{R}Carb^{R}Nif$^+$His$^+$
2	*K. pneumoniae* M5a1 Kp52 (RP41)	*E. coli* K12 SB1801	His$^+$ Km^R	5×10^{-1} 5×10^{-1}	29/29 Km^{R}Tc^{R}Carb^{R}Nif$^+$ 24/24 Tc^{R}Carb^{R}Nif$^+$His$^+$
3*	*E. coli* K12 JC5466 (RP41)	*Agrobacterium tumefaciens* 544	Tc^R His$^+$	3×10^{-3} 1×10^{-3}	52/52 Km^{R}His$^+$ 52/52 Km^{R}Tc^R 0/10Nif$^+$
4*	*A. tumefaciens* 544 (RP41)	*E. coli* K12 JC5466	His$^+$ Km^R	1×10^{-6} 1×10^{-6}	10/10 Km^{R}Tc^{R}Nif$^+$ 10/10 His$^+$Tc^{R}Nif$^+$
5†	*E. coli* K12 JC5466 (RP41)	*Rhizobium meliloti* A1	His$^+$ Tc^R	6×10^{-5} 1×10^{-5}	2/10 Tc^R 0/10Nif$^+$ 12/20 His$^+$ 0/20Nif$^+$
6†	*R. meliloti* A1 (RP41)	*E. coli* JC5466	His$^+$ His$^+$Nif$^+$ Km^R	3×10^{-2} 3×10^{-2} 1×10^{-2}	21/41 Km^R 19/20Nif$^+$ 28/33 His$^+$

Crosses were carried out on an agar surface as described in Table 1.

*Mating mixtures were prepared on plate count agar (Difco) and were incubated for 16 h before plating on selection media. Transfer frequencies were calculated relative to the number of recipients in the mating mixture before plating on the selection media.

†Mating mixtures were prepared on L agar and incubated for 16 h before exconjugant selection.

Strains: *Klebsiella pneumoniae* Kp52 (deletion covering *nif his* and *gnd*) P2 eductant of M5a1; *Agrobacterium tumefaciens* 544 *his*$^-$ Sm^R; *Rhizobium meliloti* A1 *his*$^-$ *bio*$^-$ (derivative of *R. meliloti* 41).

of both *Rhizobium* and *Agrobacterium*, suggesting that at least one *nif* gene from *K. pneumoniae* can be transcribed and translated in these bacteria.

We thank John Barzdo and Pauline Harvey for technical assistance and Dr V. Krishnapillai for carrying out the tests with *Pseudomonas aeruginosa*. We also thank Professor J. R. Postgate for constructive criticism of the manuscript. This work was supported by a grant from the Agricultural Research Council and by the Hungarian Academy of Sciences.

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Received December 18, 1975; accepted February 2, 1976

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Expression of *Klebsiella* nitrogen fixation genes (*nif*) in *Azotobacter*

NITROGEN fixation mutants have been isolated and biochemically characterised in *Azotobacter vinelandii*[1], but the absence of chromosomal gene transfer systems such as conjugation in this genus has prevented a genetic analysis of such mutants. We now report the intergeneric transfer of *Klebsiella nif* genes carried on the P-type plasmid, RP41 (refs 2 and 3), to *Azotobacter*, and their expression in this host.

The *A. vinelandii nif* mutants lacked nitrogenase molybdoprotein (AvI) activity, iron protein (AvII) activity or were pleiotropically negative for both activities. Spontanenous derivatives resistant to 25 $\mu g\ ml^{-1}$ nalidixic acid were isolated before mating with an *Escherichia coli* K12 strain carrying the *nif* plasmid RP41. Matings were done on agar plates: cultures of donor *E. coli* grown at 37 °C in Oxoid nutrient broth and *A. vinelandii* recipients grown at 28 °C in Burk's sucrose medium[4] supplemented with 2 mg ml^{-1} ammonium acetate were grown to mid logarithmic phase and mixed in a 1 : 1 ratio; 0.1-ml portions of mixtures were spread on Oxoid nutrient agar plates and incubated overnight at 34 °C. Mating mixtures and parental azotobacter cultures were resuspended and diluted in sucrose-free Burk's medium[5] and spread on selective agar media: Burk's sucrose medium containing 25 $\mu g\ ml^{-1}$ nalidixic acid to select Nif^+ exconjugants and a similar medium with 2 mg ammonium acetate and 10 $\mu g\ ml^{-1}$ tetracycline when tetracycline-resistant exconjugants were sought. Selective plates were incubated at 28 °C; all incubations were in air.

Presumptive nitrogen-fixing exconjugants were tested for acetylene-reducing ability by transfer to 5 ml liquid Burk's sucrose medium in 25-ml conical flasks. After growth at 30 °C, the cotton wool plugs were replaced by Suba-seal rubber closures. 2 ml C_2H_2 was injected and gas samples were removed at intervals for gas chromatography on a Porapak N column with a flame ionisation detector. Activities were compared with that of wild-type *A. vinelandii* grown in similar conditions.

Among the markers carried by RP41 are *nif*, *his*, resistance to tetracycline (Tc), kanamycin (Km) and carbenicillin (Carb). The transfer frequencies of *nif* and Tc to *Azotobacter* mutants are shown in Table 1. In contrast to the situation in *Klebsiella* and *E. coli*, Nif^+ exconjugants could be selected directly on plates in air. Although the transfer frequencies were low, they were significantly higher than reversion frequencies. The low observed frequencies arose at least partially from the poor growth of *A. vinelandii* on the mating plates: $\sim 10^7$ *A. vinelandii* compared with $\sim 10^8$ *E. coli* ml^{-1} when resuspended at the end of the mating period.

Fifteen of the 83 Nif^+ exconjugants listed in Table 1, including representatives of all positive recipients, were tested for nitrogenase by the acetylene test and all showed activity between 40% and 50% of that of the wild type.

Table 1 shows that nitrogenase activity was restored to three mutants which lacked AvI and one which lacked AvII activity but not to the one strain examined (UW1) which

Table 1 Intergeneric transfer of plasmid RP41 from *E. coli* K12 to *A. vinelandii*

Recipient strains	Nitrogenase activity*	Frequency of reversion to Nif^+†	Selection§	Transfer frequency‡	Exconjugant phenotype§		
UW1	$I^- II^-$	$<4\times10^{-8}$	Tc^R	3.8×10^{-7}	$28/28Km^R$,	$27/28Carb^R$,	$0/28Nif^+$
			Nif^+	$<10^{-7}$		—	
UW10	$I^- II^+$	9×10^{-8}	Tc^R	2.0×10^{-6}	$27/28Km^R$,	$24/26Carb^R$,	$25/26Nif^+$
			Nif^+	5.4×10^{-7}	$17/17Tc^R$,	$17/17Carb^R$,	$13/17Km^R$
UW100	$I^- II^+$	$<5\times10^{-8}$	Tc^R	4.0×10^{-7}		$0/9\ Carb^R$,	$9/9Nif^+$
			Nif^+	1.3×10^{-6}	$18/18Tc^R$,	$18/18Carb^R$	
UW38	$I^- II^+$	$<2\times10^{-8}$	Tc^R	1.4×10^{-7}		$27/28Carb^R$,	$27/28Nif^+$
			Nif^+	3.8×10^{-6}	$21/28Tc^R$,	$18/28Carb^R$	
UW91	$I^+ II^-$	$<3\times10^{-8}$	Tc^R	9.0×10^{-7}			$22/28Nif^+$
			Nif^+	4.1×10^{-7}	$19/19Tc^R$		

*W. J. Brill, personal communication (I and II are the molybdoprotein and iron protein, respectively, of nitrogenase).

†Reversion frequencies determined as for transfer frequencies but without donor cells. Minima signify no revertants detected with the population tested.

‡Transfer frequencies determined after plate matings (carried out as described in text) and expressed relative to the numbers of donor bacteria *E. coli* JC5466(RP41), at the end of the mating period.

§Symbols: Tc^R, resistance to 10 μg tetracycline; Km^R, resistance to 20 μg kanamycin; $Carb^R$, resistance to 200 μg carbenicillin ml^{-1}.

Table 2 Transfer of RP41 from *A. vinelandii* to *E. coli* K12

A. vinelandii donor strains	*E. coli* recipients	Selection	Transfer frequency	Exconjugant phenotype
UW100(RP41)	JC5466	His$^+$	3.2×10^{-7}	7/8 Km^R Tc^R Nif$^+$
		Tc^R	2.0×10^{-7}	1/7 His$^+$ Nif$^+$, 7/7 Km^R
		Km^R	1.4×10^{-7}	0/9 His$^+$ Nif$^+$, 9/9 Tc^R
UW100(RP41)	SB1801	His$^+$	3.0×10^{-4}	28/28 Nif$^+$ Tc^R
		Km^R	3.0×10^{-2}	28/28 Tc^R, 0/28 His$^+$ Nif$^+$
UW91(RP41)	SB1801	His$^+$	5.0×10^{-6}	8/8 Km^R Nif$^+$
		Km^R	1.0×10^{-5}	8/8 Tc^R, 0/8 Nif$^+$ His$^+$

Plate matings as described in text. Genotype of JC5466 is *trp his* recA56 *spc;* Genotype of SB1801 is *his*750 (deletion extending through *his gnd* RHA-2A) *str* λ^R *ara gal mal*A *xyl mtl* (λ^-) Symbols as in legend to Table 1.

was pleiotropically negative for both component activities. The presence of RP41 *nif* genes in one of each class of Nif$^+$ exconjugants, UW91(RP41) and UW100(RP41), was confirmed by using these strains as donors and observing transfer of *his, nif* and drug resistance markers back to *E. coli* (Table 2). Some segregation of markers occurred in these matings: exconjugants selected directly for drug resistance were mainly His$^-$ and Nif$^-$ while all but one of the His$^+$ exconjugants were Nif$^+$ and drug resistant. Such segregation has been observed in other transfers involving *nif* plasmids[3,6].

Loss of RP41 *nif* genes ought to restore the Nif$^-$ phenotype in these exconjugants. One *Azotobacter* strain UW91 (RP41) was cultured in non-selective conditions and spontaneous loss of *nif* genes occurred: After 10 subcultures in Burk's medium supplemented with 2 mg ml^{-1} ammonium acetate, all clones among 52 tested were Nif$^-$ and 28, screened for drug resistance, were resistant to Tc, Km and Carb. These drug-resistance markers, which originated on RP4, the parent plasmid of RP41 (ref 2), were stable in *A. vinelandii.*

One *Azotobacter* strain, UW100(RP41) was examined for immunological evidence for the expression of *Klebsiella nif* structural genes. Crude extracts of this strain, grown in Burk's medium, contained material which cross reacted antigenetically with antiserum prepared against purified *K. pneumoniae* nitrogenase molybdoprotein (KpI). This antigen was absent from extracts of the parental strain, UW100, grown in ammonium-limited conditions (100 µg N (as ammonium acetate) ml^{-1} Burk's medium) Material cross reacting with antiserum to purified *Azotobacter* nitrogenase molybdoprotein (AvI) was, however, present in both extracts. When these strains were grown with excess NH_4^+ ion, no such cross-reacting materials were detected in extracts prepared similarly.

We conclude from these experiments that the Nif$^+$ *Azotobacter* exconjugants carried RP41 and expressed its *nif* genes, which originated in *Klebsiella pneumoniae* strain M5a1 (ref. 2). The ability of *Azotobacter* to transcribe and translate *Klebsiella nif* genes and, in contrast to *Agrobacterium*[3], actually to make use of their products has several important consequences:

(1) The regulatory apparatus, whereby ammonia repressed nitrogenase synthesis in parents and exconjugants, is common to *Klebsiella* and *Azotobacter,* even at a molecular level For example, if glutamine synthetase (GS) is accepted as a positive activator of *nif* genes[7-9], then *Azotobacter* GS can activate *Klebsiella nif* genes.

(2) *Klebsiella nif* genes normally function only in an anaerobic environment, yet transfer to the obligate aerobe *Azotobacter* permitted their expression in air. Clearly they must have shared the oxygen exclusion processes[10] of *Azotobacter.*

(3) The possibility arises that hybrid nitrogenases, such as the active mixtures of AcI+KpII or KpI+AcII which can be studied *in vitro*[11], are functioning *in vivo,* but comparable experiments with characterised mutants of RP41 would be necessary to establish this point conclusively.

RP41 is thus potentially useful for a genetic complementation analysis of *nif* mutants in *Azotobacter.* It could also be used for the isolation of selected mutants in *Azotobacter* by introducing RP41 with characterised *nif* mutations into *Azotobacter* and preparing homogenates.

We thank J. Bardzo and J. Simkins for assistance, Dr R. A. Dixon for criticism of the manuscript and Professor W. J. Brill for the *A. vinelandii nif* mutants.

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Received December 24, 1975, accepted January 22, 1976

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Errata

In the article "Cell differentiation by 3′,5′-cyclic AMP in a lower plant" by A. K. Handa and M. M. Johri (*Nature,* **259**, 480; 1976) there is an error in Table 3. The entries under the heading 'Medium' should read . . . MM, MM+1% glucose, MM+1 µM IAA, MM+1% glucose . . . and not as printed.

In the paper "Dopamine-like renal and mesenteric vasodilation caused by apomorphine, 6-propylnorapomorphine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene", by H. J. Crumley, R M. Pinder, W. B. Hinshaw and L. I. Goldberg (*Nature,* **259**, 584; 1976) Dr Pinder's address should be : . . . Birkenhead, Auckland, New Zealand.

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nature

March 25, 1976

Abracadabra: watch it come down

It is no surprise that 'big science', that is astronomy, space research, particle and nuclear physics, is in for a lean time in the next five years in the United Kingdom. News of shrinking budgets for big science has been coming out for a year or more, generally on occasions that a particular project has "with regret" had to be abandoned. The publication last week of the report for 1974–75 of the Advisory Board for the Research Councils (ABRC) (HMSO, Cmnd 6430, 50p) only serves to give the decision a degree of finality (see following article). Nevertheless, since it is the advisory board which is charged with advising the Secretary of State for Education and Science on his responsibilities for civil science and on the allocation of the Science Budget, and since the Secretary of State generally takes that advice, it is appropriate that we should ask, at the time the report appears, how the 'big science' decision was reached, and whether it is in accord with feelings among scientists in general.

Present annual spending in the Science Budget (that is, not by government departments) is £13 million on agricultural research, £29 million on medical research, £19 million on natural environmental research, £9 million on social science research and £96 million to the Science Research Council (SRC). Of this £96 million, £7 million goes on engineering, £24 million on astronomy, space and radio, £35 million on nuclear and high energy physics, £16 million on 'science' (mathematics, physics, chemistry, biology and so on) and the rest on student support and administration. In 1980 the total expenditure is expected to be at exactly the same level in real terms, but the SRC's operations will have shrunk nearly 10% while all the other council's operations will have grown by between 6 and 10%. Within the SRC, the nuclear physics board will have had to cut back its expenditure by roughly 25%, astronomy, space and radio by roughly 16%—others will be slightly up.

The figures are actually bleaker still for big science. There has been some criticism of the growth permitted to all other activities as Buggins' turn, but the ABRC is possibly right in its assessment that increased experimental sophistication and a preponderance of young researchers probably require such financial growth just to maintain existing capabilities. Thus 25% off nuclear physics is probably more than 30% off capabilities. Finally, two major subscriptions, to CERN and the European Space Agency, account for 40% of the present expenditure on big science. These may not be renegotiable; the sum total of all this could be nearly a halving of the SRC's discretionary activities in big science by 1980.

Why is big science to be cut so severely to maintain the *status quo* elsewhere? The ABRC concedes that in these scientific fields the UK has a high reputation, and it admits that prospects of advance are "particularly good". "On the other hand, the big sciences engage relatively small numbers of research workers . . . " This is an argument with strange echoes from the most recent Commons Select Committee report—too much is in the hands of too few. It is a depressingly bad one for the ABRC to be putting forward, suggesting that, for all the agonising, it is size that has been the criterion, not quality nor even (and this would have been easier to swallow) lack of obvious pay-offs to the taxpaying public.

The ABRC was nearly unanimous in its decision. What is surprising is that the composition of the ABRC should allow it to be such. Big science expenditure is a third of all the science budget yet amongst all the research council heads, chief scientists of government departments and civil servants and academics who served on the board when the decisions were being made, only one out of nineteen had had any first hand experience of big science. Even more surprising, although the board offers seats to departmental chief scientists whose contributions to the support of non-departmental research projects can be as small as a couple of million pounds, it cannot include the Chief Scientist, Ministry of Defence, whose ministry is spending £550 million this year on research and development, but which does not sponsor non-departmental research projects by the Rothschild method. Quite apart from the experiences of the present incumbent, Sir Hermann Bondi, which would have been particularly relevant to recent discussions, it does seem wrong that a ministry which recruits large numbers of graduates and PhDs, which runs the Meteorological Office, and which co-operates in all manner of ways with outside organisations should have no formal say in the shaping of the future of Britain's civil science.

It would obviously be foolish to expect a slavishly democratic ABRC in which every interest was represented proportionally; it is clearly much better that reliance should be placed on a body of people informed by non-partisan commonsense. But there are aspects of the decision on big science and the poorly articulated reasons behind it which should at least make us wonder whether we have yet got the ABRC exactly right. ●

Research on the rack?

Chris Sherwell looks at two government documents which do not bode well for Britain's research effort

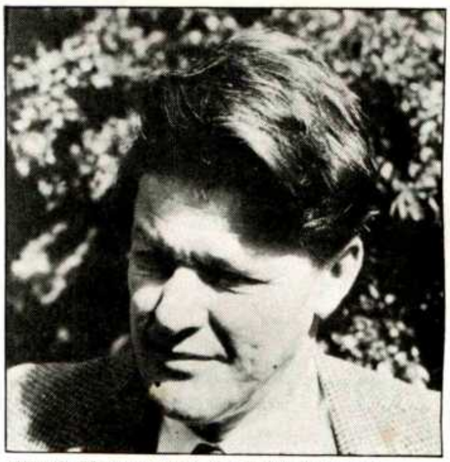
LAST WEEK, half-way between the publication three weeks previously of the Labour government's White Paper on Public Expenditure and its forthcoming revelations in the Budget, most of the country was being suitably distracted from the immediacy of its economic troubles by the resignation of Mr Harold Wilson, the Liberal Party's leadership problems and the state of Princess Margaret's marriage. Not so the scientific community. Two documents came out which directly affected its well-being. Neither can be said to contain any unexpected shocks, but the implications are that the fate of British scientific research may be hanging in the balance now more than ever: certainly the so-called "party is over" mentality has arrived.

The first document was the report of the Advisory Board for the Research Councils (ABRC)*. The report, the ABRC's second, covers the two years of its operation from the beginning of 1974 to the end of 1975; the first, published in June 1974, covered the year of its work following its formation in November 1972.

The ABRC's focus, understandably, is finance, for one of its main tasks is to advise (and its advice is usually accepted) the Minister of Education and Science, within whose departmental ambit it falls, on the allocation of the department's Science Budget. The allocation is principally amongst the Research Councils—that is, the Science Research Council (SRC), the Natural Environment Research Council (NERC), the Medical Research Council (MRC), the Agriculture Research Council (ARC) and the Social Science Research Council (SSRC)—the rest of the budget, amounting only to about 3%, is accounted for by the British Museum (Natural History) (NHM) and the Royal Society (RS).

It is through the Research Councils, which together with the University Grants Committee (UGC) constitute the "dual support system", that the government fulfils its strategic aim of maintaining a fundamental capacity for research in Britain. The Science Budget itself has grown in financial and in real terms over the last three financial years, and will do so again in the coming year (see Table 1). But for the SRC, whose share of that budget has increased up to a high point this year of more than 56%, a fall in that share is now indicated for the coming year, to 54.2%, and the expectation is for a fall to about 52% by 1980-81. More significantly, over the last two and the coming financial years, the SRC has experienced and will experience an absolute decline in real terms in the resources it receives. Annual percentage growth of 3.2% in 1973-74 gives way to negative real growth in succeeding years of 2.2%, 2.2% and, in 1976-77, 1.6%. It is a measure of the change that, in the seven years before 1973-74, growth was less than 3.2% only in 1969-70.

That change, moreover, is not regarded as a temporary phenomenon. The ABRC's guidelines regarding average annual percentage growth (also shown in Table 1) includes for the SRC a rate of −1.9% until 1980-81; and, for this year at least, the gap between the guidelines for the SRC and the other Councils will widen. As for the Science Budget as a whole, this is expected to remain at its present level up to 1980 apart from the 1.7% increase for the coming year, thus confirming both the gloomy prognosis of the White Paper on Public Expenditure and the previously indicated intentions of aiming for nil-growth.



ABRC Chairman Sir Frederick Stewart

The "keynote" of the ABRC's policy over the coming years, as its second report points out, was "redeployment away from big science (high energy nuclear physics and astronomy, space and radio sciences) to protect prospects for other natural sciences and social science" (see Figure 1). The consequence for the distribution of the SRC's resources within its own constituent parts is that nuclear physics and ASR (astronomy, space and radio) are the ones to suffer (see Figure 2). Thus, following the SRC's 1974 decision that it was unable to finance a major new £20-millions radio telescope at Jodrell Bank, and its 1975 decision (in light of the German decision approving Germany's PETRA accelerator project) that international finance would not be forthcoming for its proposed EPIC accelerator, it now looks as though existing big projects are in jeopardy. The most important is NIMROD, the 13-year-old 7000 MeV proton synchrotron at the SRC's Rutherford Laboratory, and a final decision is expected soon. A decision has already been taken regarding the closure of the NINA Synchrotron Radiation Facility.

**Second Report of the Advisory Board for the Research Councils* (HMSO, Cmnd. 6430).

Table 1 Distribution and annual growth of the Science Budget

	1973–74			1974–75			1975–76			1976–77			Average annual % growth guidelines to 1980-81
	£ million	% of total	Real annual % growth	£ million	% of total	Real annual % growth	£ million	% of total	Real annual % growth	£ million	% of total	Real annual % growth	
ARC	16.824	12.0	4.0	15.114	10.2	−3.7	13.171	7.7	−0.6	18.33	8.5	3.3	1.8
MRC	25.664	18.3	2.7	26.144	17.2	−1.7	29.022	16.9	−1.0	37.36	17.3	2.7	1.7
NERC	15.796	11.2	4.4	16.066[1]	10.5	−3.0	19.252[1]	11.2	0.2	26.05[1]	12.1	2.3	2.0
SRC	71.429	50.9	3.2	83.665[1]	55.1	−2.2	96.713[1]	56.4	−2.2	117.19[1]	54.3	−1.6	−1.9
SSRC	5.854	4.2	8.6	6.767	4.4	5.7	8.749	5.1	6.8	11.18	5.2	2.0	2.0
NHM	2.647[2]	1.9	23.1	2.581[2]	1.7	−2.1	2.873	1.7	1.0	3.86	1.8	—	—
RS	2.170	1.5	3.5	1.499	1.0	2.3	1.733	1.0	—	1.98	0.9	−1.7	1.0
Total	140.384	100.0	3.9	151.665	100.0	−2.1	171.513	100.0	−1.3	215.95	100.1	1.7	

Transferred funds are not included.

[1] SRC and NERC figures include an element of the costs of dispersal, not included in the Science Budget.

[2] Includes cost of buildings borne on Department of Environment Vote (1973–74, £0.9 million; 1974–75, £0.6 million).

Britain's position undermined?

All of which tends to reinforce the view that Britain's position in the "big science league" is likely to be undermined, which some may argue is a bad thing. The ABRC, however, has found itself obliged to take a somewhat different view. Aside from the government's own exhortation that cuts be made, and the fact that these are most readily achieved by axing the biggest and thus most expensive projects, two strands of thought appear to inform the ABRC's overall disposition regarding big science. The first is the general policy one described in its 1974 and 1975 reports: that, apart from the exigencies of overall redeployment, substantial priority also had to be accorded to the SRC's Engineering and Science Boards over the needs of big science, a priority reflected in Figure 2. In 1974-75, fully 60% of the SRC's budget went to big science, while only 11% and 15% respectively went to the Engineering and Science Boards. (Big science's 60% is equivalent to about 33% of the total Science Budget).

It was an imbalance the ABRC thought should be redressed, and one argument its Chairman, Sir Fred Stewart, voiced in justification, apart from the official one of maintaining a "general national scientific capability" overall, was that to support engineering, an activity central to Britain's economic performance, was to improve the chances of more resources for big science later when, with improved economic performance, those resources would be available. No assumptions about such a recovery are being made at the moment, however: the proportion of the total science budget going to big science is projected to fall rapidly from 33% to 27% in 1980-81.

The other important element in ABRC and SRC thinking is inescapable. The SRC faces certain crucial constraints on its capacity to redeploy its funds to the degree it might perhaps wish. These include important international commitments, to the European Space Agency, the European Science Foundation, CERN and so on. These commitments, as they stood last year, amounted to 26% of the SRC's expenditure—and were equivalent to 44% of the Nuclear Physics Board's funds and 24% of the ASR Board's. It is these sorts of commitments, of course, which must be considered in accounting for the sizeable shares going to big science, just as it is the often colossal cost of SRC projects as a whole which must be weighed in any judgments that are made about the majority share of funds which historically goes to the SRC rather than other Research Councils. Both of these factors, by their nature, must aggravate the impact on British science of the cuts now in store.

Other Research Councils are less harshly treated. The ABRC says it has decided to use extra funds made available by the increase for 1976-77 "in such a way as to reinforce the redeployment policy" it has adopted, bearing in mind that the increase was only a "temporary departure" from the nil-growth trend. It stresses that there is no modification in its policy of distributing resources secured from savings in big science roughly proportionately to the other Research Councils and to the Engineering and Science Boards of the SRC. As for maintaining the basic research "floor", which it endeavours to do through an improving co-ordination with the UGC, the ABRC says there is "likely to be a need for greater selectivity and concentration of resources on the part of the Research Councils".

White Paper on Defence

The trouble is, British scientific research is not starting to go through a particularly lean period just because of the reductions in funds available to big science, to the SRC, or even to the less badly hit ARC, MRC and NERC. A second document released last week, also in the wake of the Public Expenditure White Paper, is rather more difficult to interpret, but it tends to reinforce the pessimistic view. That document was the government's annual White Paper on Defence† It is worth remembering, if only for the sake of perspective, that the Department of Education and Science's Science Budget in 1975-76, for instance, accounted for only about 12½% of the total government expenditure on research and development—a level that has in fact tended to fall in recent years (it was closer to 17½% in 1971-72, for example). In 1975-76, when the Science Budget was around the £150 millions mark, government research and development spending in what is classified as "higher and further education" was approximately £116 millions, in "trade, industry and employment" it was about £246 millions—and in defence it was over £550 millions.

Not all of the figure ascribed to defence, of course, goes to pure research. A research and development figure of £702 millions in the latest White Paper compares with last year's forecast (at the same price level) of £669 millions and represents 12% of the overall defence budget of some £5,600 millions Of this £702 millions, £107 millions is forecast expenditure on research, while the rest goes to "development of approved systems"; of the £107 millions, about two-thirds, the White Paper says, relates to work, including exploratory development, in direct support of projects in the equipment programme. The remaining one third relates to longer term research. Another £22 millions is to be spent on research work "directed to civil objectives".

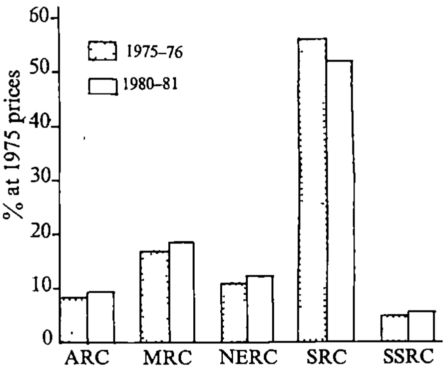


Fig. 1 Research Councils' percentage shares of the Science Budget 1975–76 and 1980–81, as reflected in in 1975 guidelines

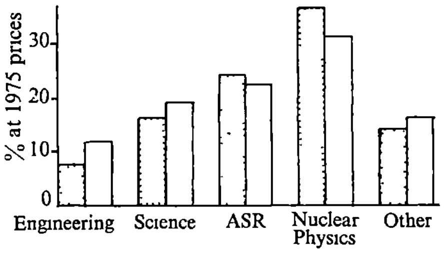


Fig. 2 SRC Boards' percentage shares of the SRC Budget 1975–76 and 1980–81, as reflected in SRC 1975 Forward Look.

The breakdown of the £702 millions is not very revealing: £258 millions is attached to military aircraft, £74 millions to guided weapons, £97 millions to "other electronics", £73 millions to ship construction and underwater warfare, £58 millions to "ordnance and other army", £139 millions to "other research and development"—and £3 millions to meteorological research and development. What is revealing, though, is the degree to which the overall cutbacks in defence are forcing the closure of research establishments attached to the ministry Economies in the Ministry of Defence's research programme, which is organised into 18 major fields (aerodynamics, electronics, armaments and so on) and is done in defence-related industries and university research laboratories as well as the ministry's own establishments, have forced the ministry not only to decide on the second stage of its rationalisation of its research and development programme but also to proceed with "and where practicable to accelerate" it.

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†*Statement on the Defence Estimates 1976* (HMSO, Cmnd. 6432).

As a result, a number of sites will be closed "in due course", some "as soon as possible" but most within five to seven years. They include the Chemical Defence Establishment outstation at Nancekuke in Cornwall, reportedly associated with the development of nerve gas and CS riot gas; future levels of research and development on "defence against chemical and biological warfare", carried out at the parent Chemical Defence Establishment and the Microbiological Research Establishment (both at Porton), are to be reviewed "with the object of making significant economies". They also include the Admiralty's engineering, oil and materials laboratories, and one site of another of the Admiralty's laboratories, as well as a site of the Explosives Research and Development Establishment. In addition, there will be "a further cut on extramural research with industry and the universities where for the time being we shall have to sacrifice some of the longer-term work". Concentration of activities in the field of explosives will enable the work of closed sites to be carried out at the Atomic Weapons Research Establishment and the Royal Armament Research and Development Establishment. Other work will be concentrated as a result of the naval laboratory closures Redundancies are inevitable, and could amount to many hundreds.

The underlying aim of the rationalisation is to complete "the framework of a research and development organisation based on four main systems establishments—sea, land, air, and underwater—complemented and supported by a number of technology establishments". The aim, in other words, is similar to the comparable one in the civilian area which the Rothschild White Paper foreshadowed, namely, better and more efficient co-ordination of government-backed scientific research and development. While the cuts in defence appear to be encouraging the pace of such a rationalisation, though, the redeployment policy of the ABRC as implemented through the SRC is looking increasingly like an issue separable from the other main focus of the ABRC report, the more universal application of the "customer-contractor principle".

"Customer-contractor principle"

Government departments which act as regular "customers" for Councils to which they "contract" research are represented on both the relevant Councils themselves and the ABRC through the comparatively recent institutions of their own Chief Scientific Advisers (CSA). The "customer-contractor principle", outlined by Rothschild in respect of research and development conducted in accordance with objectives formulated by the departments themselves, has already been in operation for some time within the Ministry of Defence through its CSA; its contractor is the Procurement Executive. The principle should have been implemented within three years (that is, by next week) by certain other departments: thus, some of the funds previously lying with the ARC, MRC and the NERC (contractors) should by now have been transferred to their respective customer departments for the departments' own use, which could perhaps be outside the Councils through private research establishments.

The ABRC report is not discouraging about progress in this respect, at least regarding the ARC and MRC But it acknowledges the complexity of the case of the NERC and its customers, which stems from the sheer diversity of its activity: "There will continue to be some difficulties", the report says, "to which the Council and probably the Board itself will need to give careful thought" But while the ABRC recognises these matters, it studiously avoids the related question of co-ordination amongst the CSAs themselves. That co-ordination, such as it is, is currently pursued largely through the ABRC itself, for at the moment there is no CSA for the government as a whole: the last one, Sir Allan Cottrell, has not been replaced, and the presence on the ABRC of Sir Keith Berrill, head of the Central Policy Review Staff which advises the Cabinet on strategy, was affirmed as being in his personal capacity only.

These issues are not likely to disappear. But for the moment the main questions surround the government's financing of British scientific research. The country's research effort, while not a party issue, is a political issue insofar as it has to be resolved at the political level. At the moment, however, it manifests itself to the scientific community only as a matter to be rather mechanically resolved at Research Council and ABRC level; and is largely a question of slicing up a financial pie whose size is fixed by Treasury *diktat* Not only is the size of the pie itself not a major object of contention; the manner in which it is sliced was, as Sir Fred Stewart vigorously insisted last week, the product of consensus. The degree to which the claims of participants in the various branches of scientific research are recognised is unclear, but, in the case of the budget handled by the SRC, it is not obvious that its size, both absolutely and in relation to the other Councils, is an issue thoroughly discussed any lower than the SRC itself.

Thus, whether or not it is true, as the House of Commons Select Committee on Science and Technology recently argued, that SRC funds are directed largely at the behest of a small charmed circle of scientists, it seems clear that that alleged circle may now be losing (if indeed it is conducting) any fight to preserve the share of total funds it has quietly thought itself entitled to in the past. The difficulty, of course, is that in a new nil-growth era, when even the country's defence effort is under scrutiny, Britain's scientists as a whole, like the rest of the country, will over the next few years be losing as well. □

USSR

Broom at the top

Following the disasters of Soviet agriculture during the last Five-year Plan, the USSR Minister of Agriculture Dmitrii Polyanskii has been relieved of his post. Polyanskii was not re-elected to the Politburo at the end of the recent Twenty-Fifth Party Congress, when his Ministry faced sharp criticism, notably from Aleksandr Lyashko, the Prime Minister of the Ukrainian SSR; he did, however, retain his seat on the Central Committee of the Party. Now his dismissal as scape-goat for his Ministry appears to be a tacit acknowledgement that the criticisms were well-founded, and that more than "abnormal weather conditions" were responsible for the agricultural short-fall

The new Minister of Agriculture, Valentin K. Mesyats, is a graduate of the Moscow Agriculture Institute. From 1965–1971, he was Minister of Agriculture of the Russian SFSR. Since then he has held the post of Second Secretary of the Communist Party of Kazakhstan (one of the Republics most involved in the Virgin Lands scheme) He is a Member of the Central Committee, but not a member of the Politburo.

Two newcomers to the Politburo fill the places left by the departure of Polyanskii and the 80-year-old Anastas Mikoyan. They include Dmitrii Ustinov, who has for some 10 years been closely connected with the Soviet's armaments programme, including nuclear arms and missiles. This appointment gives two seats on the 16-man Politburo to military interests—the other being held by Minister of Defence Marshal Andrei Grechko.

Vera Rich

Siting the sun set

In the United States, the Energy Research and Development Administration (ERDA) has faced numerous complications over its solar energy plans. And, as Colin Norman reports from Washington, problems remain

IT HAS been several years since a major political scrap broke out in the United States over the location of a major new research facility, for the good reason that there haven't been many new facilities established recently. But in the next few months every state is expected to compete for a scientific prize—a Solar Energy Research Institute, to be established by the Energy Research and Development Administration (ERDA).

The location of the institute won't be the only source of dispute, however, for last week ERDA announced plans for the facility which suggest that it will be a much more modest enterprise than many of its proponents had envisaged.

Plans for the institute have been through a long, and difficult, gestation period. Approved by Congress as part of a bill passed in November 1974, the institute, known by its acronym SERI, was expected to be a major national laboratory for planning and carrying out government research on solar energy. Congress left ERDA to decide exactly what the role and scope of SERI should be, and it also asked the National Academy of Sciences to assist ERDA in the planning.

When the bill was approved, it was generally assumed by SERI's congressional sponsors that the institute would rapidly evolve into the focal point for the government's solar energy programme, and that it would develop into a major facility along the lines of ERDA's nuclear laboratories such as Oak Ridge or Los Alamos. And that assumption was reinforced by the Academy, which last October recommended that SERI should be provided with a budget of about $50 million and a staff of 630 by 1980.

Modest effort

ERDA officials also hoped to get moving quickly on a large facility but, following a series of disputes with the White House culminating in the resignation of a senior ERDA administrator, the agency last week announced plans for a much more modest effort. The plans, set out in a formal invitation for public and private institutions to bid for a contract to establish SERI, envisage a modest beginning over the next three years, with no absolute guarantee that the institute will ever develop into ERDA's leading solar energy laboratory.

ERDA, in short, has asked for proposals to establish a solar energy management and research team at an existing institution, with a budget of between $4 and $6 million for the first year, rising to $10–20 million in the third year. In addition, ERDA has asked the bidders to propose a nearby site for a larger, separate facility into which SERI could move at a later date if necessary. The idea is to provide for deliberate, phased growth possibly—though not definitely—to a major national laboratory in the early 1980s.

Under ERDA's direction, SERI will provide analysis and planning for research and development programmes on solar energy, ranging from direct use of sunlight for heating and cooling buildings to electricity generation, wind power, and bioconversion. In addition, SERI will perform some research and development itself. Dr Robert Hirsch, acting assistant ERDA administrator for solar, geothermal and advanced energy, said last week that he expects a contractor and location to be chosen in December, and that SERI will begin operations on January 1, 1977 A decision on whether or not SERI will move to a large, permanent site is expected in two or three years, he added.

Cautious approach

ERDA arrived at that pared down proposal after the White House Office of Management and Budget (OMB) had vetoed its earlier plans for a more ambitious facility. OMB, understandably, argued that ERDA should proceed with caution, moving to a large facility only if the need for a major enterprise is demonstrated. OMB's reluctance to move with dispatch on SERI, and on other parts of the solar energy programme, led to the resignation in January of John M. Teem, Hirsch's predecessor in charge of ERDA's solar energy efforts.

The cautious approach embodied in the proposals is likely to provide considerable ammunition for solar energy proponents, who have long argued that the solar programme has been held back in the past few years while funding for nuclear energy has been growing by leaps and bounds Though government expenditures on solar energy and development have grown from $200,000 in 1972 to an anticipated $116 million next year, the total proposed for the nuclear programme next year amounts to nearly $3,000 million. Solar energy proponents are particularly fond of pointing out that the breeder reactor alone—which, like some uses of solar energy isn't expected to make any contribution to energy supplies until the 1990s—amounts to $880 million next year.

Nevertheless, since SERI is likely to be a prestigious institute which will attract top-level talent and perhaps some associated industry, there is likely to be fierce competition between the states for the contract The competition in fact started several months ago when several states set up committees to plan their strategies for securing SERI. ERDA has also been inundated with inquiries from interested institutions.

The last tussle over the location of a major research facility in the United States was during the Johnson Administration, when the National Accelerator Laboratory was up for grabs. In the end, that facility was located in Illinois because, according to widespread speculation, Johnson needed the support of Illinois Senator Everett Dirksen, then Senate minority leader, on several key items of legislation. ERDA, however, is anxious to avoid any suspicion that such squalid political deals will decide the site for SERI, and it has insisted that the location will be chosen on strictly scientific and other "factual" criteria.

ERDA taken aback

ERDA officials were therefore somewhat taken aback when President Ford told a Colorado energy symposium last September that Arizona, New Mexico and Florida are the leading contenders to house SERI. Ford, moreover, told officials from Florida's Brevard County recently that their region would receive "excellent consideration" as the site for the institute.

Be that as it may, Hirsch said last week that SERI could be located anywhere in the United States, and that he expects to receive proposals from all 50 states. He noted that the institute will be performing a range of work on solar energy and that some technologies (generation of electricity from wind, for example) don't need large quantities of sunlight. It should not therefore be assumed that SERI must be located in the sunny southwest, he said.

Proposals must be submitted to ERDA by July 15, a site selection board composed of ERDA officials will evaluate them, and a final decision will be taken by ERDA administrator Robert C. Seamans, Jun. in December. ◻

CANADA

Eldorado radiates Hope

David Spurgeon reports from Ottawa on the Port Hope affair

As a result of continuing public concern about radioactive contamination in the Port Hope area of Ontario, a federal–provincial task force drawn from different departments of the national and Ontario governments was formed late last month to supervise a clean-up of the area. The force will also help the Atomic Energy Control Board (AECB) to discuss the significance of radioactivity in other locations in Canada.

The formation of the task force was announced by the Federal Minister of Energy, Mines and Resources, Alastair Gillespie, after documents were tabled in the House of Commons reporting on the Port Hope situation. These revealed a total of 109 locations in 25 areas of Canada where radioactivity is known or suspected.

Radioactivity from radon gas was discovered last year in a Port Hope school and at several other sites, including private homes. It came from waste disposal from Eldorado Nuclear Limited, which had recovered radium in the 1930s and 1940s from pitchblende-radium-silver ores mined in the Northwest Territories. The abundant pitchblende (uranium) content was largely discarded at that time because it was thought to be of little value. The radium extracted was used for cancer treatment and in luminous dial painting.

During that period, wastes were disposed of at Port Hope in piles on and close to the Eldorado plant. Toxicity and radiation hazards standards were not then well established. Some of the waste is believed later to have found its way to various parts of the town in the form of fill that was trucked about.

Wastes were also sold to a company in the United States for recovery of metals, which involved shipping the wastes about, and some of the contamination apparently came from spills *en route*, or from storage. A building programme by Eldorado Nuclear Limited may also have resulted in contamination of rubble, fill and reclaimed building materials.

During the Christmas festive period last year, a street-by-street survey of the whole town of Port Hope was made by the AECB and the Ontario health ministry. Samples of air were collected for analysis when abnormal radioactivity levels were found.

At one private home, air samples taken outside the house showed radon concentrations ranging from 200 to 400 times the acceptable level for continuous exposure. Contaminated fill had been used to fill a ravine behind the house. The health ministry has closed a school, and several families have vacated their homes.

A number of recommendations have already been carried out to remedy the situation in Port Hope. The task force's immediate priority is to complete the investigation and clean-up in Port Hope. The AECB has opened a public information centre there and has assigned two staff officers and two vehicles to the investigation, and plans have been made to carry out an aerial radiometric survey.

If nothing else it seems likely that any future plans to install nuclear capacity on the shores of lake Ontario will be subjected to close scrutiny in the wake of the Port Hope affair. All the same, it remains a source of worry that minor warnings about Port Hope had been given over many years before the latest developments occurred. ☐

SWEDEN

Generating nuclear heat

The use of nuclear energy, as Wendy Barnaby indicates in this report from Stockholm, is a sensitive issue in Sweden

Mr Ralph Nader, the American consumer expert, has said that the Swedes' nuclear energy programme is hindering their ability to work for world peace. At a meeting of environmentalists in Gothenburg, Mr Nader said he was disappointed with the Swedish Prime Minister, Mr Olof Palme, who had in his opinion been too quick to accept arguments for a rapid expansion of nuclear power. (Mr Palme's Social Democratic government plans to have 13 reactors in operation by 1985). If Sweden concentrated on nuclear energy it could not refuse to back other countries making the same choice. But, Mr Nader maintained, the only way to stop the proliferation of nuclear weapons is to stop the spread of nuclear energy for peaceful purposes as well.

Meanwhile, Mr Björn Gillberg—often called "Sweden's Nader"—is waiting for a court decision in one of his many battles against nuclear power. The case, fought on behalf of a local environment group by Gillberg and a lawyer, Gunnar Michelson, against the State Power Board, concerns permission to operate reactors three and four at the Ringhals plant on the west coast. Technicalities have put the case into the court of appeals of the Water Court, whose jurisdiction is limited to considering whether the reactors could damage their water environment. The only aspect of the plant able to be discussed has therefore been the release of cooling water into the sea.

If permission is given for the reactor to be operated, 175 cubic metres of cooling water per second—the largest release in the world—will flood into the sea through pipes so large that trucks can be driven through them. The environmentalists contend that the effect of a court decision in favour of operation would be to cause a certain number of deaths from the water's radioactivity, and that the court has no right to make decisions which kill people. The State Power Board agrees that every year of operation would cause some cancers and genetic defects, although its estimation of their number is smaller by a factor of eight than the environmentalists'.

As the reactors are already being built, it is highly unlikely that permission to operate them in some form or another will be refused. Mr Gillberg is of course aware of this, but maintains that nuclear power should be fought on all fronts, no matter how small the odds of winning may be.

Not that Mr Gillberg and his friends should be unhappy with the latest opinion poll on nuclear energy. Fully 54% of the sample wanted no more than the five reactors already built—and this position was taken by 41% of Social Democratic supporters. Only 34% of the sample wanted more than the present five: the rest did not know. More surprising, however, was that 75% of the sample preferred to curb their standard of living in order to avoid an increase in energy use, rather than to raise their standard of living and accept the consequences of using more energy. And the majority comprised 70% of the Social Democratic respondents. The vital question, though, is not the divisions in opinion polls, but the effects they have, in this election year, on public policy. ☐

IN BRIEF

Marine pollution

Delegates to the Inter-Governmental Maritime Consultative Organisation, meeting in Acapulco this week to discuss marine pollution from ships, will consider UK Government reports which survey national and international counter-pollution arrangements and outline UK contingency plans to tackle major oil spills in territorial waters. While claiming that existing UK measures have already proved partially successful in dispersing a 300-tonne spill in the English Channel last November, the reports stress the need for improved international cooperation. Discussions between France, Norway and Britain are already under way.

Anders move

In a surprise announcement, President Ford last week nominated William A. Anders, Chairman of the Nuclear Regulatory Commission (NRC), to be ambassador to Norway Anders, a former astronaut, established a reputation as a tough administrator during the first year of the commission's activities as watchdog over the nuclear industry Ford indicated that he would nominate Marcus A. Rowden, a former general counsel to the Atomic Energy Commission and a member of the NRC, to succeed Anders.

Cyclamate view

A committee of cancer experts in the USA has failed to reach a definitive conclusion about whether or not the banned artificial sweetener cyclamate is a carcinogen The committee, appointed by the National Cancer Institute to help the Food and Drug Administration decide whether to lift the ban on cyclamate, noted that most of the tests leading to a significant increase in tumours in cyclamate-treated animals have employed dubious procedures or used strains of animals which have a large incidence of spontaneous tumours, but said the results were a cause for concern

UK chemical industry

Following last week's National Economic Development Office report urging the UK chemical industry, now at "a decisive point", to reassess existing rates of investment and bring forward planned projects so as to improve international competitiveness beyond 1978, an independent publication from the Chemical Industries Association (CIA) has called on the Government to boost managerial confidence by easing the price and profit controls cutting into available revenue at a time when investment demands are three times higher than in 1974 The CIA report says that the industry's plans to spend £2,800 millions on new plant and equipment over the next three years indicate a willingness to meet projected market demands. The difficulties faced by the industry were simultaneously underlined by the publication of a third report, showing that several leading chemical companies have been trading at a real loss over the past three years

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My lady nicotine

THOMAS H. JUKES

THE delights of tobacco were recorded in the panegyric "My Lady Nicotine" by James Barrie Perhaps he should instead have used the word "nitrosamine", as the *grande dame* who lurks in the fragrant weed *N′*-nitrosonornicotine (NNN) was identified by Hoffmann and coworkers in unburned tobacco at levels ranging from 2 to 89 parts per million Most samples contained about 4 p p m. This may be compared with about one four-hundredth of this level of nitrosopyrrolidine in bacon, a commodity that is now figuratively as well as literally in the frying-pan for its content of nitrosamines NNN induces multiple pulmonary adenomas in mice, but just how much of the carcinogenic effect of cigarettes is caused by nitrosamines is not known. The smoke from one cigarette contained 0 14 microgram of NNN, which is non-volatile, and other nitrosamines that are volatile. The well-televised habit of chewing tobacco is familiar to all baseball fans, but I have seen no epidemiological studies on pitchers

A few weeks ago my lady nicotine (or nitrosamine) came knocking at my door in the guise of a letter from Mr William D Hobbs, the chairman of the board of the R J. Reynolds Tobacco Company I was, he told me, a leader in my community, and, therefore, I would want to know about a technological achievement consisting of a new cigarette, which "draws free and easy" (*sic*). If I were not a smoker, he said, I might wish to refer this offer of two packages of cigarettes to someone who I thought might be interested I told him I had a better idea. I believed that all advertising of cigarettes should stop, and that direct mail advertising to start someone in the habit of smoking was particularly pernicious

My answer to this came from a company executive, Mr Crohn, presumably assigned by Mr Hobbs to respond to anti-cigarette cranks He sent a letter, and various pieces of literature favouring cigarettes One item was an article reprinted from *Executive Health*, a well-advertised publication that sends out appeals for funds to support an Institute for Orthomolecular Medicine. The article was entitled "The Case Against Tobacco Is Not Closed. Why smoking may not be 'dangerous to your health'" Good health, executives! Mr Crohn's letter told me that if advertising of cigarettes were to cease, then "the public would never know that low tar and nicotine cigarettes, which many persons desire, are available in the market place".

The phrase "which many persons desire" rang a faint mnemonic bell of the first casket scene in *The Merchant of Venice*—written before men were persons and coffins were caskets In this scene, the gold casket has the inscription "Who chooseth me shall gain what many men desire" Upon reading this, the Prince of Morocco says, "Why, that's the lady all the world desires her" Was Mr Crohn a crypto-Shakespearean? Did he mean many persons desire My Lady Nicotine? But then, in the play, the casket is opened to reveal

> "A carrion Death, within whose empty eye
> There is a written scroll!"

The scroll, I mused, perhaps said, "Warning The Surgeon General has determined that cigarette smoking is dangerous to your health".

We shall never know The warning was written in letters too small to read.

correspondence

Allergic reactions to laboratory animals

SIR—There is a growing concern about allergic reactions in laboratory workers who handle animals but little factual evidence is available. The British Society for Allergy and Clinical Immunology appointed a working party to investigate this subject and here we report preliminary findings, which give some idea of the size of the problem.

We used a questionnaire in two laboratories, one industrial and the other non-industrial, but the results were closely similar, so here we amalgamate them 474 individuals were questioned. Salient points were, that of this population,

- 23% had had one or more symptoms occurring repeatedly within 12 hours of animal contact
- 9% had had chest, 17% nasal, 10% eye and 11% skin symptoms
- 4% had had to stop working with animals
- after two years of exposure the incidence of allergy did not increase significantly with further exposure
- a family history of allergy was present in 22% of those who reported symptoms, 19% of those who did not. This surprisingly small difference calls into question the usefulness of a pre-employment family-history screen, and is at variance with results from a recent survey in the USA (Lutsky I. I., and Neuman, I., *Annals Allergy*, **31**, 201 (1975)).

We regard the figure of 23% as disturbingly high, higher than those reported recently from the USA (two surveys gave 15% and 11%), and we are starting a much larger survey to investigate factors which may alter the incidence of allergic reactions in exposed populations.

Yours faithfully,
GEOFFREY TAYLOR (CHMN.)
G. E. DAVIES (SEC.)
R. E. C. ALTOUNYAN
H. MORROW BROWN
A. W. FRANKLAND
J. MORRISON SMITH
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Volume publishing

SIR,—I write to draw attention to, to underline, and to generalise from, the exquisite review (March 4, page 82) of the *Handbook of Perception,* which I have not seen. Dr John Mollon lambasts non-editing, a feature not confined to the publishers of the *Handbook,* and unfortunately rife in the field of science publishing

Progressive specialisation and illiteracy make it clearly more and more difficult for any one author to cover a field in depth: hence the mushrooming of Editored volumes during the last decade or two. A publisher approaches a Name, offers a percentage out of all proportion to the services rendered (which frequently consist in no more than drawing up a list of contributors and acting as a relay station for the receipt of typescripts), and informs the Inland Revenue at the appropriate time.

The contributors are also offered a percentage out of all proportion to the services rendered: I know of a case where the typist was paid more than the author. They are often up-and-coming youngsters who will not heed the warnings of us older hacks. I always point out to younger colleagues that fixed payments are to be avoided like the plague especially if they are made after publication: inflation eats into the terms but the selling price of the book can protect the publisher and editor. I also stress the naïveté of the notion that they will derive any kudos: later references are almost invariably made to the Editor-so-called, with the author's name sunk in Lethe. The crowning insult occurs when a generous publisher sends along 25 reprints, and so ensures a reduction in sales.

It seems to me that there is a case for an agreed code of good conduct to protect the interests of (young) authors who probably cannot afford the luxury of membership of authors' protective associations—which can, in any case, do little more than offer advice. Moreover, if Editors started spelling their designation with a lower-case e, the interests of authors might begin to be guarded, and one of the last relics of child labour expunged from society.

Yours faithfully,
R. A. WEALE

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London, UK

Alternative refrigerants

SIR—The letter from Birks and Leck, (Correspondence, March 4, page 8) avers that while we can do without aerosol cans, our civilization cannot function without refrigerators. It is then suggested that, if we do not choose to give up refrigerators, we may have to continue tolerating atmospheric pollution by chlorofluorocarbons, even if we ban aerosol cans.

The implication that we are faced with such an awkward choice is not, I think, correct. It is true that we can prevent release of chlorofluorocarbons into the atmosphere by aerosol cans in only one way: ban aerosol cans. It is an essential part of their operation to release the gas they contain.

This is not true of refrigerators, which normally release their refrigerant gas only when junked. This release could be almost completely prevented by

- a law making it an offence carrying a heavy penalty to junk a refrigerator without first having a suitable public or private (licensed) agency remove the refrigerant, or
- much better, requiring a meaningfully large deposit to be left when a refrigerator is purchased and rebating that sum, plus inflation related interest, only upon presentation of proof that the refrigerant has been duly recovered by the designated agency.

We can, in short, keep refrigerators and save our atmosphere, even if it becomes necessary to discontinue using aerosol cans.

Yours faithfully,
F. A. COTTON

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The pace of life

SIR—Hermits can communicate with everyone in their community by standing still (February 19, page 557 and March 18, page 188). But what about pedestrians in downtown Tokyo (population in excess of 10^7)? Can anyone confirm that they walk at almost 2 m s^{-1}?

Yours faithfully,
RALPH A. LEWIN

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news and views

Suppression of foreign synapses

from Jeremy Brockes

WHEN a foreign nerve is implanted into a normally innervated skeletal muscle, it grows out over the surface of the muscle fibres but does not synapse with them. If, at this juncture, the normal innervation is removed by cutting or crushing the nerve, then the foreign nerve will now form functional synapses on the denervated fibres. The original nerve eventually regenerates and synapses again with the muscle. This results in muscle fibres which have been 'tricked' into accepting innervation from two sources. The subsequent fate of these dually innervated fibres is currently of great interest to neurobiologists, since one can ask the basic question of whether the foreign innervation is recognised, and somehow suppressed or removed in favour of the original. Such a mechanism could be of considerable importance in trying to account for the great specificity and plasticity of connections that is exhibited by the nervous system.

The principal proponents of such a mechanism have been Mark and his colleagues who have provided evidence that in both goldfish eye muscle (Marotte and Mark, *Brain Res.*, **19**, 41, 53; 1970) and adult salamander muscle (Cass *et al.*, *Nature*, **243**, 201; 1973), the foreign innervation can be functionally suppressed. Furthermore, they have claimed that the suppressed synapses are not retracted, but remain on the muscle fibre and appear normal when examined in the electron microscope (Marrotte and Mark, *Brain Res.*, **19**, 53; 1970). The assay for function in the goldfish eye muscle was indirect, involving behavioural testing of the eye movements, and their interpretation of the results is currently controversial since a repetition and extension of these experiments has led to the conclusion that the foreign innervation is not suppressed (Scott, *Science*, **189**, 644; 1975). Meanwhile, in several other situations, including one where the different synapses were formed on a neurone, the dual innervation has proved perfectly stable with no evidence of suppression, even after several months (Frank *et al*, *Nature*, **247**, 375; 1974 and Purves, *Nature*, **256**, 589; 1975). But in a timely contribution (this issue of *Nature*, page 350), Yip and Dennis have provided clear electrophysiological evidence that suppression does take place in dually innervated fibres of the adult newt By intracellular recording from individual muscle fibres, they have been able to show that transmitter release from the foreign nerve terminals is reduced as a consequence of re-innervation by the original nerve. Specifically, the amplitude of the synaptic potential decreases during the first two months following re-innervation. The synaptic potential is derived from a number of packets, or quanta, of transmitter that are recruited synchronously by stimulating the nerve. By analysing a number of fibres at different times during the period of suppression, Yip and Dennis have shown that the number of packets recruited (the 'quantal content') declines while the amount of transmitter in a single packet does not change significantly. This is a most important insight into the suppression process, since it characterises it as a presynaptic effect on transmitter release rather than a postsynaptic effect on, for example, the chemosensitivity of the muscle membrane.

An important question, which has yet to be answered, concerns the eventual fate of the suppressed synapses. Are foreign synapses withdrawn from the muscle when the original nerve returns, or do they remain structurally intact but functionally suppressed, as suggested by Mark and his colleagues? When, after six months, the original nerve was interrupted for a second time, Yip and Dennis found that the re-establishment of functional transmission through the foreign nerve appeared to occur more rapidly than after the initial operation. This suggests that the foreign nerve axons remain in relatively intimate contact with the muscle, but does not necessarily mean that foreign synapses are present In an analogous situation during normal development, it is known that mammalian skeletal muscle fibres are multiply innervated before birth (Redfern, *J Physiol., Lond.*, **209**, 701; 1970; Bennett and Pettigrew, *J. Physiol., Lond.*, **241**, 515; 9174); when this multiple innervation is eliminated during development, the extra synapses are completed retracted. Although this process is not identical to that observed by Yip and Dennis, it does illustrate that competitive interactions between synapses can lead to the removal of extra innervation.

Meanwhile, one wonders what rules govern the operation of suppression, and why it did not take place in several other situations Is it, as Yip and Dennis suggest, a question of retaining in the adult newt a faculty which is normally confined to development, or is it perhaps related to the way that foreign and original synapses are distributed along the length of the muscle fibres? It would be interesting to determine whether the foreign nerve in Yip and Dennis's study can become the correct one when the reciprocal experiment is performed in its own muscle. The most helpful insights, however, will probably come from an analysis of the mechanism. One would like to know if the interaction has to occur between synapses that are relatively close (of the order of microns) or whether it can occur over distances of the order of millimetres. If the latter is so, one might suspect that the muscle fibre plays a direct role in the process. An informative experiment could be to take a muscle that is only partially re-innervated by the orginal nerve, and determine if the suppressive influence can act on foreign synapses present on adjacent fibres that were not re-innervated. A second major question, which I have considered above, concerns the effect of the interaction on the anatomy of the suppressed synapses. If we knew the answers to these two questions, then the number of plausible mechanisms would be reduced.

I do not think that we can distinguish these possibilities with the available evidence, but some should be clearly excluded by an anatomical study. Other questions, such as the role of activity in the two nerves, and in the muscle fibre, also seem approachable. This whole problem illustrates the advantage of working on the neuromuscular junction—a preparation with which one can ask a basic question about synaptic function, and hope to answer it at several levels. Furthermore, there is every reason to believe that these answers will prove relevant to the experimentally less accessible situation of synapses in the central nervous system. ☐

Helping hand to nuclear technology

from Andrew Holmes-Siedle

As Townsend commented in a recent review (*Nature,* **258,** 293; 1975), in many universities, solid-state physics is taught in too ideal a fashion, as if real solids consisted of perfect arrays of atoms. While the existence of defects may be acknowledged, their importance or impact is not given its true weight. As a result, physicists often see the physics of defects as a fringe area of little importance. The commonly held belief is that colour centres and other invisible point defects are academic curiosities, confined to those near-useless materials, the alkali halides. So the report of a distinguished working party of the American Physical Society (Vook *et al., Rev. mod. Phys.,* **47,** Suppl 3; 1975) which states precisely the importance which small defects could have in nuclear technology and even makes a reasoned estimate of the possible cost of ignorance in this branch of physics is especially welcome.

The task at hand is to find out what will happen to the materials that constitute the structures and containment systems for the massive nuclear reactors (fission and fusion) which will supply most of our energy in two decades' time. Over the anticipated 20-year life of these reactors, some parts will receive fast neutron fluences in the range 10^{23} to 10^{42} fast neutrons cm^{-2}. The effect of this irradiation will be that every atom will have been moved from its lattice position about a hundred times and this constitutes a very serious case of radiation damage. The costs of ignorance in this field, it is claimed, could be as high as $10,000 million over the coming decade

One might ask whether all the basic radiation damage problems have not been worked out in the first development of power reactors. But the neutron flux levels envisaged in future fission reactors (fast breeder power reactors) are orders of magnitude higher than for present ones; the fluxes of particles expected to irradiate the walls of fusion reactors, moreover, are even higher and more varied in type Also, past research in radiation damage, though intensive, has only scratched the surface of a field of great depth and subtlety. In fact, present basic knowledge of defects is too superficial even for the needs of today's reactor designers.

The working party's estimate of future costs due to ignorance stem from a vision both of the immense overdesign which often results when knowledge is inadequate and of the inefficiency which results from "large-scale specific R and D testing imposed by limitations of basic knowledge". It sets out to delineate and dispel this limitation with detailed recommendations for research work which, although not costed in the report, could very probably be done for much less than $10,000 million.

In the report, entitled *Radiation Effects on Materials* and one of a series on "Physics Problems relating to Energy Technology", the radiation effects field is split into ten main aspects. The ideas and numbers were hammered out during a ten-day seminar at Brookhaven. The problems of the different classes of defects (small, extended and so on) are first discussed; then the complexities of theory and simulation of damage by neutrons and some special effects (hydrogen, helium, surface effects) are described mainly in relation to metals. Finally, the three other solid state forms of greatest interest are discussed —semiconductors, superconductors and insulators Reactor technology has its own section. In each case, a succinct description is followed by reasoned recommendations, while the main object —to point to specific areas in which the physicists can help the reactor designer—is kept in mind throughout.

The complexity of some of the physical problems can be illustrated by the void problem (see, for example, Bullough and Nelson, *Physics in technology,* **5,** 29, 1975) At temperatures in the 500–800 °C range, the conventional recombinations between atom vacancies and interstitial displaced atoms are disturbed, defects aggregate instead of recombining and the material swells A volume increase of 15% is not unusual The aggregated defects can be observed as interstitial loops, which are not new to us but the vacancies, at high damage levels, form a structure which is completely new to physics, namely an aggregate of vacancies large enough to form a visible void in the lattice. Such a structure introduces very great complications into the physical model of damage, since it represents an internal surface Among other practical consequences of its existence, the new surface can act as a sink for impurities and for gaseous transmutation products trapped in the material. Their presence will also affect strength, but it is hard to predict exactly what effects will result from a given change in the composition of a material. Without a predictive method, physicists cannot help the materials engineer to develop or select more radiation tolerant materials

The economic usefulness of the physicist here is in his ability to change what might otherwise be a near-random search for better materials into one in which principles for the needed improvement of materials can be stated and tested in the least expensive way Once the best possible material has been developed, the physicist's power to predict its properties after 20 years of bombardment may then tip the scales in the question of the reactor's economic viability Without these data the designer might, using a necessary conservatism, design a cart-horse of a reactor, immensely strong mechanically but poor at making power. There will also be a need, not mentioned in the report, for a common scientific/engineering language which will facilitate communication between specialists.

Because a magnetically-confined fusion reactor, unlike a uranium reactor operates at high voltage, thermonuclear power reactors (if they are ever developed) will require a variety of strong, long-lived insulators. The report dwells on our knowledge of radiation damage in the alkali halides, and our lack of it in the oxides. But it missed an excellent argument for extra effort in these fields. Whereas the damage problem is greatest in the structural metals, the techniques by which damage can be understood in conductors are more limited than those which can be employed for dielectrics, mainly because optical and magnetic resonance spectroscopy cannot be used There seems, however, to be a good chance that models for damage processes which are common to both types of material (for example the escape of defects from damage spikes, their diffusion in the lattice and their addition and loss from voids and loops) could be developed to a high level of sophistication using dielectric samples and the models could then be corrected as necessary for the case of metals and applied to the serious problem of degradation in strength.

Another little-known fact which is clearly documented in the report is the difficulty of simulating in a short time the 20-year torture which reactor structures will receive Neutron sources of the required flux and energy (14 MeV for fusion, and about 1 MeV for fission) do not exist for the damage range (up to say 500 displacements per atom) which we need to explore. For many purposes, beams of heavy ions can simulate neutron damage well enough. For example, about 10^{16} 7.5 MeV tantalum ions cm^{-2} in vanadium foils at 700 °C produce voids which are not dissimilar to those produced by 10^{22} fast neutrons cm^{-2} ($E > 1$ MeV). The latter irradiation would take months in the most powerful fission reactor while the former takes hours or minutes in an accelerator.

The working party makes two pleas —that the solid-state physicist should be brought more intimately into the nuclear programme and that, to save money, money will have to be spent on simulation machines and basic research. Good arguments are offered as to how overdesign through ignorance could undermine economic viability. Now, it is to be hoped that the agencies planning our future in the energy field will consider this message and implement it. Perhaps a similar call by European scientists, especially confirming the financial theme, would drive the point home to their own governments. ☐

Filamentous phage assembly

from P. J. G. Butler

The filamentous bacteriophages of *Escherichia coli* are a family of viruses consisting of a single-stranded circular DNA molecule, encapsidated into a very long, narrow and flexible particle (reviewed by Marvin and Hohn, *Bact. Rev.*, **33**, 172; 1969). Unlike other bacteriophages, they are neither lysogenic nor do they establish a lytic infection, but rather a productive infection resulting in the slow secretion of progeny virus during a comparatively long period. This poses particular problems during virus release, which occurs without either lysis of the cell membrane or the budding off of regions of this membrane as with lipoprotein coated virions. The solution lies in a series of specific interactions between the viral coat protein and the cytoplasmic membrane of the cell, which are now beginning to be described.

The virus particles contain predominantly a single type of coat protein of about 5,000 daltons. Marvin and Wachtel (*Nature*, **253**, 19; 1975) have analysed the X-ray scattering from oriented gels of one of these bacteriophages (Pf1) and were able to fit this with a model in which the protein molecules are fully α helical and are themselves arranged helically in the particle, with the axes of the α helices lying almost parallel to the particle axis. This results in a tight interlocking of the coat protein molecules, giving a high degree of protection to the DNA while allowing flexibility of the particle. The sequences of the coat proteins are compatible with such a structure and Nakashima *et al.* (*Nature*, **253**, 68; 1975) showed that the hydrophobic central region could readily form stable α helices which could interact in the appropriate way, while there is a slight excess of basic residues at the C terminus, which could be at the inside of the protein coat and help to neutralise the DNA phosphate residues.

Although the attachment of the phage to the host cell, on or near the few molecules of attachment protein per particle, appears to be relatively straightforward, the transport of the viral DNA both into and out of the cell through the cytoplasmic membrane involves more complex mechanisms. The DNA uptake during infection requires DNA synthesis in the cell, during which Marco, Jazwinski and Kornberg (*Virology*, **62**, 209; 1974) found the single-stranded viral DNA to be converted into the double-stranded replicative form (RF). As the DNA is drawn into the host cell, the viral coat protein becomes incorporated into the cytoplasmic membrane and Smilowitz (*J. Virol.*, **13**, 94; 1974) showed that it is reused during the subsequent maturation of progeny virions, being incorporated dispersively into their protein coats, and thus leading to an economy of protein synthesis.

After the establishment of the infection, with viral protein and DNA synthesis in the host cell, the assembly of progeny virus occurs. Initially the new virion DNA strands are assembled into a nucleoprotein complex with the protein product from gene 5, which is not the virus coat protein. These nucleoprotein complexes are again long, flexible particles and the protein molecules are probably helically arranged. However, Pratt, Laws and Griffiths (*J. molec. Biol.*, **82**, 425; 1974), while demonstrating their formation *in vivo*, found that these particles are much less stable than the mature virions, in which the gene 5 protein has been replaced by the coat protein. They suggested that the intracellular coat serves to protect the DNA until it acquires its final coat while being extruded through the cell membrane, to which the coat protein has already bound, and then recycles onto fresh DNA.

The site of the interaction between the phage coat protein (of M13) and the cytoplasmic membrane has been examined by Wickner (*Proc natn. Acad. Sci. U.S.A.*, **72**, 4749; 1975). He found that ^{125}I-labelled antibody raised against detergent-solubilised coat protein would bind specifically to both the intact phage and the coat protein in the membrane. Using this antibody to assay the coat protein, initially of the infecting virus, he confirmed that RF formation was necessary for the transfer of the protein from the incoming virus to the cytoplasmic membrane of spheroplasts, this process taking about 10 min and leaving the protein available for reuse in progeny virion assembly. After about 40 min, further coat protein begins to accumulate in the membrane as it is synthesised. Both the parental and the newly synthesised protein are similarly located in the cytoplasmic membrane, with the antigenic sites only detectable on the outside of the membrane and being masked if the spheroplasts are inverted by sonication. This observation implies that the protein must occupy a position in the membrane irrespective of whether it is inserted from the outside, during initial infection, or from the inside by *de novo* synthesis. Such rapid transport across the membrane is in marked contrast to the slow rate of flipping of phosphatidyl choline, which Kornberg and McConnell (*Biochemistry*, **10**, 1111; 1971) showed to have a half-time for equilibration of about 6.5 h, suggesting that some specific mechanism may be involved for its insertion through the membrane.

Further evidence for specificity in the insertion of M13 coat protein into the membrane, whether from the inside or the outside, comes from Wickner's observation that the antibody reaction is similar with either spheroplasts or intact cells. Since these cells do not release their periplasmic enzymes, the coat protein must have some special

A hundred years ago

Several letters have appeared in the *Daily News* calling attention to the fact that on Sunday week red snow was observed to have fallen in several parts of the country—at Forest Hill and Streatham in the south of London, at Reading and at Thurston in Norfolk. This phenomenon was observed in ancient times, and is referred to by Pliny; in modern times it has been frequently observed in all parts of the world, and is familiar to Arctic explorers. The phenomenon is generally ascribed to the presence of an algae, *Protococcus nivalis*.

from *Nature*, **13**, March 23, 415; 1876 localisation which allows access of the antibody.

In spite of the similar antigenic reaction of the coat protein, whether in the intact phage or incorporated into the cell membrane, Nozaki *et al.* (*Nature*, **259**, 335; 1976) suggest that the protein conformations show substantial differences. They examined the circular diochroism spectra of intact phage (f1) and of its coat protein in aqueous solution with various detergents or dissolved in micelle-forming lysolipids. The spectrum of the protein in the intact virus is quite unlike that of other proteins in simple aqueous solution, for while it shows the peak wavelengths characteristic of an α-helical chain, the intensities of these peaks are surprisingly different This unusual intensity distribution can be explained by the bunching of the chromophores which will occur in the packing of more than 2,000 protein molecules into a single virus particle. With this correction, the spectrum is consistent with the entirely α-helical conformation for the protein previously suggested by Marvin and his colleagues. The spectra of the amphiphile-solubilised protein are all similar to each other and different from that of the protein in the virus in a way which suggests less than 50% α-helix content This change is not, however, consistent with a significant part of the polypeptide having a random coil conformation and Nozaki *et al* suggest that the transition may be from α helix to β conformation. Such a conformation would be compatible with the observed dimeric state of the protein in detergents and with the amino acid sequence around residues 26 to 36.

This structural change does not essentially contradict the similar antigenicity, since Wickner cites preliminary evidence that the antigenic site is in the first eight amino acid residues from the N terminus. In the model proposed by Marvin and Wachtel, this region of the polypeptide chain is on the surface of the virus particle and it is not the region suggested to undergo the structural transition But this gross rearrangement of secondary structure is unique among the mechanisms so far proposed for virus assembly and the interest of this phage protein–cell membrane system should encourage further study. ☐

Kelp, abalone and sea otters

Robert M May

BEFORE being all but exterminated by fur traders in the 18th and 19th centuries, the sea otter (*Enhydra lutris*) occupied a range around the northeast rim of the Pacific Ocean from the northern Japanese archipelago, through the Aleutian Islands, and along the coast of North America as far south as Baja California. Natural populations have persisted mainly in remote parts of the original range, particularly in the Aleutians and parts of southeastern Alaska. Recently, however, with the enactment of protective legislation and the reintroduction of sea otter populations off the coasts of Oregon, Washington, British Columbia and parts of central California, the species is making a comeback along the Pacific coast of North America.

Although this fact is gratifying to conservationists, it is less pleasing to those involved with abalone fisheries. Adult sea otters in captivity have been found to eat 20–25% of their body weight daily, and benthic invertebrates (such as sea urchins or abalone) are the main component of this diet. Several studies have shown a decrease in sport and commercial abalone fisheries following the arrival of sea otters into previously unoccupied areas in California.

The dominant role played by sea otters in determining the structure and dynamics of nearshore communities is demonstrated by several recent ecological studies Estes and Palmisano (*Science*, **185**, 1058–1060; 1974) compared islands in the western Aleutians where sea otters have occurred naturally for at least 20–40 years (the Rat Island group) with islands lacking otters (the Near Islands). They found many floral and faunal differences between the lower intertidal marine communities on these islands· the Rat Islands have an almost complete mat of benthic brown algae (kelp), with both sessile filter-feeding invertebrates (barnacles and mussels) and motile herbivorous invertebrates (sea urchins and chitons) being inconspicuous, small and scarce; the Near Islands are heavily grazed by dense populations of sea urchins and chitons. The density of barnacles, mussels, sea urchins and chitons, respectively, averaged around 4.9, 3.8, 8, and 1 m^{-2} at the Rat Islands, and 1,200, 720, 78, and 38 m^{-2} at the Near Islands.

Dayton (*Fish Bull*, **73**, 230–237; 1975) has followed this with a very detailed study of the competitive interactions between three kelp canopy "guilds" in a community at Amchitka Island, Alaska, in which herbivorous invertebrates have been largely removed from shallow waters by sea otters. This work confirms that sea otters are extremely important in maintaining the structure of shallow water algal communities, and tempts Dayton to the evolutionary speculation that, by reducing herbivorous predation, the sea otters permit a competitive differentiation of niches whereby the several kelp species coexist.

All these people are in agreement that, as sea otters become more established along the California coast, there should be a pronounced increase in the abundance of nearshore kelp. Dayton cautions against accepting this as evidence of the "natural" order of things, and he emphasises that the once-abundant giant sea cow (*Hydrodamalis gigas*) must have had important consequences to the kelp populations, that undercut any present day speculation as to the evolutionary consequences of kelp competition. Indeed, Dayton conjectures, it is likely that by consuming invertebrate herbivores (particularly sea urchins) "the sea otter was indirectly responsible for the high productivity of large algae necessary to maintain the sea cow populations." This conjecture is supported by the overlap of otter and sea cow populations in the Pleistocene.

This story is typical of many, where layer after layer of complication unfolds as one seeks to elucidate the structure of the pristine ecosystem, as it was before first sea cows and then sea otters were removed. Dayton gives a general warning against drawing evolutionary morals from partially reconstructed systems, because one or more crucial species may have become extinct at a time which seems distant in human memories, but is recent on an evolutionary time scale. ☐

Glaciology's grand unsolved problem

from J Weertman

OVER the past two decades our understanding of the behaviour and the motion of glaciers, ice shelves, and ice sheets has progressed and increased very nicely. We know enough now to recognise a grand glaciological problem that remains to be solved. The West Antarctic Ice Sheet is this problem Actually it is a set of inter-related problems. The base of this ice sheet over a major part of its area is 0.5 to 1 km below sea level. A major fraction of its bed would remain below sea level if the ice sheet were removed and isostatic rebound took place. How then did this ice sheet form? Why does it remain in existence? Is it growing or disintegrating at the present time? The ice in one half of the West Antarctic Ice Sheet is draining primarily through very fast moving ice streams into the Ross Ice Shelf. These ice streams apparently are not centred over any deep, fiord-like channels cut into the bedrock, and therefore could not have existed in their present position for any great length of time. The velocity of ice in the ice streams, about 1 km yr^{-1}, is comparable to that of the ill-understood surging glaciers. (Surging glaciers temporarily move, over a 1 to 2-yr period, at velocities that are one to three orders of magnitude larger than a normal glacier velocity.) Why do very fast moving ice streams form at the periphery of the West Antarctic Ice Sheet? Why do they move so swiftly? Is their existence a premonitory phenomenon to a large scale surge of the whole ice sheet? Are the ice streams the start of a process that may cause the West Antarctic Ice Sheet in the near future to flow very rapidly and to discharge about one-third to one-half of its total volume of ice into the oceans over a short time period of the order, say, of 100 years?

Geological evidence that was studied by Denton, Armstrong, and Stuiver (in *Late Cenozoic Glacial Ages* (edit by Turekian) 267, Yale University Press, 1971) and by Denton and Borns (*Antarctic J*, **9**, 167; 1974) indicates that before 10,000 years ago the West Antarctic Ice Sheet was much larger and covered the area, now below sea level, presently occupied by the Ross Ice Shelf. The Ross Ice Shelf, although about 300 to 500 m thick, is afloat. Thus, the edge of the ice sheet has retreated about 1,000 km with an average rate of retreat of the order of 100 m yr^{-1}.

It is not likely that this large scale retreat was produced by any change in the climate. There is at present a net accumulation of snow on top of both the West Antarctic Ice Sheet and the Ross Ice Shelf; in fact, the accumulation rate is larger on the ice shelf than on the ice sheet. Studies of the ice cores from drill holes in both the ice sheet and the ice shelf have produced no evidence at all of any long lasting period of widespread ablation that would have caused the shrinkage of the West Antarctic Ice Sheet. A more likely explanation of the retreat is that it was caused by the large rise in sea level, 100 to 130 m, that occurred when the ice sheets in the northern hemisphere melted at the end of the last ice age According to theory (Weertman, *J Glaciol*, **13**, 3; 1974) the size of an ice sheet whose base is below sea level is a very sensitive function of changes in sea level Whether the ice sheet can even exist over a long period of time is dependent upon the sea level.

The geological evidence of fossil coral-reef terraces in the Barbados (Broecker *et al.*, *Science*, **159**, 297; 1968) indicates that 120,000 years ago the sea level was 6 m higher than it is today Old coral-reef terraces in New Guinea (Chappell, *Geol. Soc. Amer. Bull.*, **85**, 553; 1974) also support this conclusion. A 6 m rise in sea level requires the total removal (and melting) of ice from an ice mass of about the size of the West Antarctic Ice Sheet or the Greenland Ice Sheet and its emplacement into the world's oceans. This observation of a 6 m higher sea level 120,000 years ago suggests that the West Antarctic Ice Sheet can largely disappear under conditions that do not lead to the destruction of the East Antarctic Ice Sheet. The destruction of the latter ice mass would have raised the sea level by another 40 to 50 m.

Terence Hughes of the University of Maine has strongly advocated the view (and put forward supporting evidence) that the West Antarctic Ice Sheet is still disintegrating (*J geophys. Res*, **78**, 7884; 1973). He thinks that the rate of retreat of the edge of the ice sheet where it joins the Ross Ice Shelf is of the order of 70 m yr^{-1} This rate of destruction of the ice sheet would cause the sea level to rise with a velocity of the order of 0.5 mm yr^{-1}. The long term increase in mean sea level occurring now has been estimated from various measurements to be 1 mm yr^{-1} (Lisitzin, *Sea Level Changes*, 183, Elsevier, Amsterdam, 1974). Thus, a slowly occurring destruction of the West Antarctic Ice Sheet could account

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Metallic glazes

from Robert W. Cahn

A NUMBER of alloys based on transition metals—notably nickel and iron —can be made in the form of glassy ribbons by ultra-rapid quenching of a molten jet on a rapidly spinning roller (see *Nature*, **252**, 100, 1974; **259**, 271, 1976). Iron-based glassy ribbons containing chromium have the further distinguishing feature of being extremely corrosion resistant. These materials, however, have to be in the form of thin (0 05 mm), narrow (1–2 mm) ribbons, and there is not a great deal that the most ingenious designer can do with them Much interest therefore attaches to a report of a novel method that allows the surface of bulk material to be converted into glass without affecting the interior.

A group of metallurgists at the United Technologies Research Center in Connecticut, led by Bernard H. Kear and Edward M. Breinan have described to the American Society for Metals how a continuous carbon dioxide laser can be used to "glaze" the surface of nickel or cobalt-based alloys. The Center is linked with a factory that makes aircraft engines, and the implications of the choice of alloys are clear enough.

A finely focused beam from the CO$_2$ laser is passed over the surface of the bulk material; the surface is rapidly melted and as rapidly refrozen as the hot spot moves on. A cooling rate exceeding a million degrees per second was claimed, during resolidification, and this suffices to generate a glassy (non-crystalline) phase at the surface The alloy compositions used are not specified it would be interesting to know whether they contain metalloid solutes such as P, C, B, Si; as do the alloys used to make glassy ribbons. It is particularly worth noting that the underlying crystalline grains do not act as nuclei for the resolidifying surface film.

The investigating team reports that the surface glaze is harder than the base alloy, and adds that it is "potentially more capable of resisting corrosion than the original alloys": the cautious choice of words leaves one uncertain whether the potential had been realised.

This is not the first time that a laser has been used to effect rapid quenching from the melt. Laridjani, Ramachandrarao and Cahn (*J. Mater. Sci.*, **7**, 627; 1972) used a stationary beam from a pulsed ruby-laser to produce metastable crystalline phases on the surface of Ag–Ge alloys, and Elliott, Gagliano and Krauss (*Metallurgical Trans.*, **4**, 2031; 1973) used a pulsed neodymium laser to create a continuous series of solid solutions in a range of Cu–Ag alloys which are only partially miscible in equilibrium. The United Technologies metallurgists have improved on this earlier work in two ways: they examined alloys based on transition metals which, unlike copper and silver-based alloys, are apt to form glasses on melt quenching, and they used a moving beam from a continuously acting laser to scan the surface. What is not clear from the report is whether it was feasible to make a series of overlapping passes in such a way to cover a surface entirely with a glazed layer If this can be done, the industrial potential is very considerable; it should in principle be possible to "coat" even the inside of a vessel with the aid of a guided moving mirror. There is also potential for the design of wholly or partially glazed bearing surfaces.

for the present rate of rise of the mean sea level.

It would be difficult to measure a yearly shift of the position of the edge of the West Antarctic Ice Sheet of the order of only 100 m. The thickness of ice at the junction of this ice sheet with the Ross Ice Shelf is at least 500 m. The position of the grounding line certaintly cannot be found with an error limit smaller than the ice thickness. It seems clear that a great deal of field data must be collected on such things as ice thickness on both the Ross Ice Shelf and the West Antarctic Ice Sheet before the question can be answered whether this ice sheet is disintegrating or growing and at what rate.

Many data have been collected now, at least for the Ross Ice Shelf (see Robin, *Nature*, **253**, 168; 1975). These and new data have been analysed by Thomas (*Nature*, **259**, 180; 1976). He thinks that at the grounding line the Ross Ice Shelf is thickening at the high rate of 1 m yr^{-1}. This rate of thickening then leads to his startling conclusion that the position of the edge of the ice sheet at least at one location is advancing at the very fast rate of 1 km yr^{-1} Thomas cautions that this result is not expected to be typical He believes that the apparent current thinning of the ice sheet nearer its centre is a delayed response to an earlier retreat of the grounding line.

So now a controversy exists about whether the West Antarctic Ice Sheet is growing or retreating and whether perhaps it is starting to surge. It will take a lot of new field data from the West Antarctic Ice Sheet as well as theoretical analyses to settle the question. □

Errors in lipoprotein metabolism

from a Correspondent

FAMILIAL hypercholesterolemia is an inborn error of metabolism in which affected individuals show a marked elevation in plasma cholesterol, progressive coronary, cerebral and peripheral vascular occlusive disease associated with accumulation of cholesterol in atheromatous plaques. Death from myocardial infarction often occurs before the age of 30. Cholesterol accumulation, which is the most striking biochemical change seen in these patients could arise because of increased synthesis or because of a failure of response by the liver to feedback controls on that synthesis by plasma levels of cholesterol. Some recent findings have gone a considerable way to defining at least one cellular abnormality carried by some patients with this disease. It does seem, however, that the clearly identified clinical phenotype may hide several different abnormalities at the cellular level and therefore what I report here refers only to a proportion of affected individuals. Nevertheless, the recent results of Brown, Goldstein, Steinberg and their colleagues provide exciting pointers to one probable cause of this disease.

A genetic abnormality characteristic of the disease trait in some instances is carried by skin fibroblasts established from biopsy material. It was shown by Brown and Goldstein (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2162–2166;1973), that normal skin fibroblasts exposed for about a day to foetal calf serum made deficient in lipoproteins accelerate cholesterol biosynthesis by activation of a key enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase. The normal fibroblasts respond, through regulation of the level of activity of the enzyme, to inhibition of cholesterol biosynthesis by addition of lipoprotein. The response is rapid, occurring over a few hours. In contrast to the normal behaviour, skin fibroblasts from homozygous patients with hypercholesterolemia do not respond to lipoprotein and cholesterol synthesis continues unabated in lipoprotein-supplemented serum (*Proc. natn. Acad. Sci. U S.A.*, **70**, 2804–2808; 1973). Brown and Goldstein reasoned that the effect of lipoprotein on the fibroblasts must be mediated firstly by attachment of lipoprotein to surface receptors followed by internalisation of lipoprotein and a terminal effect on cytoplasmic events involved in cholesterol biosynthesis. On the basis of binding data of lipoprotein to normal or hypercholesterolemic fibroblasts, these workers proposed (*J. cell. Physiol.*, **85**, 425–436; 1975), that the specific block was in the lack of high affinity surface receptors in the latter cells. The binding data were, however, collected by treating cells with labelled lipoprotein at 37 °C, that is, under conditions in which other events, quite apart from the initial binding of lipoprotein molecules to their specific cell receptors, would be expected to take place. It seems probable that both binding and internalisation of lipoprotein molecules would occur, by analogy with the well studied capping and envelopment of lymphocyte surface immunoglobulin after combination with bivalent antibodies at elevated temperatures but not in the cold (see Raff, *News and Views*, **259**, 265–266; 1976). The binding data of Goldstein and Brown probably sums the levels of lipoprotein bound at the cell surface and intracellular lipoprotein, and it becomes difficult to distinguish a block in internalisation of bound lipoprotein molecules rather than in the binding process itself.

Stein *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 14–18; 1976) have reassessed this problem, and propose that it is indeed the internalisation step that is grossly defective, at least in the cells studied by them. They carried out experiments in which the binding of radioiodinated lipoprotein to fibroblasts was measured at 0 °C The differences compared with normal fibroblasts were small at best, not greater than about two-fold Essentially all the bound radioiodinated lipoprotein was released from both normal and hypercholesterolemic cells by mild trypsin treatment without disruption of the cells. Therefore, it was concluded that these measurements refer accurately to cell surface binding and the latter cells seem to be almost normal in this respect. Of course, it is certainly possible that this relatively small decrease in lipoprotein binding may be important in the physiological response of the cells to lipoprotein, for example by the specific loss of a class of cell surface receptors important for the subsequent events of lipoprotein action on cholesterol synthesis. But by far the most striking effect was seen in the amounts of labelled lipoprotein present inside the cells after incubation at 37 °C. This was measured after treatment with trypsin to remove surface located molecules. The normal cells contained from 14–115 times as much labelled material as the hypercholesterolemic cells, measured under the same conditions.

What is the nature of the "receptors" for lipoprotein in the fibroblast surface membrane? This is not known with any certainty, nor indeed whether there are specific "receptors". The lipid moiety of the lipoprotein could react in some way directly with other lipids of the cell membrane. It seems, however, that the sites at which lipoprotein molecules become associated with the membrane may be critical for exertion of the biological effect on cholesterol biosynthesis, implying recognition of these sites by lipoprotein. At high concentrations of endogenously added lipoprotein, the level of internalised material in hypercholesterolemic fibroblasts may rise to comparable levels to those found in normal fibroblasts exposed to more moderate concentrations. Yet there is no accompanying inhibition of sterol synthesis. Goldstein *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1092–1096; 1975) therefore suggest that the interaction with specific receptors on the membrane is a necessary step in the overall control mechanism, and presumably this interaction is also critical in internalisation. If this turns out to be the case, the identification of the specific membrane components responsible is clearly of great importance in defining the ultimate defect in hypercholesterolemia. ☐

Who killed T7?

from Rich Condit

For people interested in control of translation, for those interested in phage protein–host membrane interactions, or for people who simply enjoy molecular whodunnits, the history of research into the abortive infection of male *E coli* by bacteriophage T7 makes a rather good story.

T7 is a virulent phage which grows only on female *E. coli* (Makela *et al.*, *Ann Med. exp. fenn.*, **42,** 188; 1964). When male *E. coli* are infected with T7, the cells are killed as is normal in a T7 infection, but no cell lysis occurs and no phage are produced. The initial probe into the molecular biology of the abortive infection indicated that the phage DNA entered the male cell and was completely transcribed, but that only early T7 proteins were produced In addition, the wild type F factor (PifA$^+$B$^+$) could be mutated so that the block in translation was either partially (PifA$^-$B$^+$) or completely (PifA$^-$B$^-$) relieved (Morrison and Malamy, *Nature new Biol*, **231,** 37; 1971). The hypothesis which emerged to explain these phenomena maintained that during a normal infection, T7 makes two translational control factors, each required for translation of a discrete class of late T7 mRNAs, and that the F factor encodes two proteins, each of which specifically sabotages one of the T7 coded factors. Because of this hypothesis, T7 has been regarded as a model system for studying translational control of protein synthesis.

In the past few months, several reports have appeared which render most of the translational control hypothesis untenable, and suggest alternative explanations for the abortive infection. One of the most important facts emerging from these studies is that, in contrast to initial findings, it now seems that T7 mRNA synthesis is abnormal in infected male cells. Analysis of phage RNA synthesis in male cells by pulse labelling, by hybridisation, and by translation reveals that although synthesis of early T7 mRNA is normal and synthesis of some late mRNA is close to normal, many late phage RNAs are synthesised in substantially reduced amounts, or not at all (see for example Britton and Haselkorn, *Virology*, **67,** 264; 1975; Condit and Steitz, *J. molec. Biol.*, **98,** 31; 1975; Young and Menard, *J. molec. Biol.*, **99,** 167; 1975). In fact, it seems that T7 infection of male cells results in a generalised paralysis in synthesis of macromolecules (RNA, protein, and DNA) rather than simply a block in protein synthesis. In short, the infection appears normal until late phage protein synthesis just begins (by which time some late RNAs have been made in substantial amounts) and then the infection simply dies.

Attempts to reconstruct the translational control hypothesis *in vitro* have met with only partial success. First, ribosomes from uninfected male and female cells are identical with respect to their ability to synthesise T7 proteins. Second, ribosomes from uninfected cells are fully capable of translating late T7 mRNAs, demonstrating that no phage-induced alteration of host ribosomes is necessary for translation of late T7 mRNA. Finally, though some groups have been unable to detect any significant defect in ribosomes extracted from T7 infected male cells (Condit and Steitz, *op. cit.*; Ponta *et al.*, *Eur. J. Biochem.*, **59,** 261; 1975; Young and Menard, *op. cit*), others have observed that these ribosomes are reduced in activity in an *in vitro* system by a factor of 3–5, relative to ribosomes from uninfected cells or cells undergoing a productive T7 infection (Yamada and Nakada, *J. Virol.*, **16,** 1483; 1975; Blumberg *et al.*, *J Virol.*, **17,** 94; 1976). The defect is generalised, that is, the ribosomes from infected male cells are defective regardless of the mRNA used in the test, the defect seems to be reversible by addition of a trypsin-sensitive, non-dialysable fraction from cells containing healthy ribosomes (Blumberg *et al.*, *J. Virol.*, **17,** 94, 1976), and the defect becomes apparent during infection of wild-type male cells at approximately the same time that everything else goes wrong (Yamada and Nakada, *J. Virol.*, *op. cit.*)

In summary, what remains of the translational control hypothesis is that the ribosomes in male cells seem to sustain a certain amount of damage as the T7 infection aborts.

In the search for an explanation for the cataclysmic death of the infected cell, it has been discovered (Britton and Haselkorn, *Proc. natn. Acad. Sci. U.S A*, **72,** 2222; 1975; Condit, *J. molec. Biol.*, **98,** 45; 1975) that at precisely the same time that macromolecular synthesis comes to a halt the T7 infected male cell becomes "leaky". There is a rapid loss of intracellular nucleoside triphosphates into the surrounding medium, the cells lose the ability to accumulate glutamine, and they become abnormally permeable to orthonitrophenylgalactoside Furthermore, the appearance of these permeability defects depends upon both the presence of the F-factor gene(s) controlling the abortive infection, and on phage protein synthesis.

It is tempting to tack ribosome damage onto the list of things that might go wrong if membrane function is tampered with, and to formulate a "permeability hypothesis" to explain the abortive infection Perhaps, because of the action of the F genes, a T7 protein misbehaves in the male cell and membrane function is rendered aberrant as a result. Macromolecular synthesis might be expected to cease because of a change in the intracellular ionic conditions and a decrease in the availability of small molecules A recent study by Blumberg, Mabie and Malamy (*J. Virol*, **17,** 94; 1976) however, questions the notion that membrane damage, ribosome damage, and the overall paralysis of macromolecular synthesis are causally related.

Blumberg *et al* have compared leakage of ATP with activity of ribosomes using four types of T7-infected cells: F$^-$ (productive infection, lysis at 30 min), PifA$^+$B$^+$ (wild-type male, infection aborts at approximately 8 min), PifA$^-$B$^+$ (mutant male, infection aborts at approximately 13 min) and PifA$^-$B$^-$ (mutant male, productive infection). They find that PifA$^-$B$^-$ cells behave exactly like F$^-$ cells, that is they contain healthy ribosomes wrapped in healthy membranes. Whereas PifA$^+$B$^+$ and PifA$^-$B$^+$ cells show virtually identical leakage of ATP, however, ribosomes extracted at 8 min after infection are defective in PifA$^+$B$^+$ cells and normal in PifA$^-$B$^+$ cells. These observations indicate that the sudden paralysis of protein synthesis and the damage to ribosomes observed during infection of a wild-type male cell cannot be accounted for solely in terms of leakage of ATP. The PifA$^-$B$^+$ cell begins

Table 1

	Infected cell type			
	F$^-$ (female)	PifA$^+$B$^+$ (male)	PifA$^-$B$^+$	PifA$^-$B$^-$
Infection phenotype	Permissive	Abortive	Abortive	Permissive
Phage protein synthesis *in vivo*	Ceases with cell lysis (30 min)	Ceases ~ 8 min	Ceases ~ 13 min	Ceases with cell lysis (30 min)
Ribosomes extracted at 8 min damaged?	No	Yes	No	No
Leakage of ATP at 8 min?	No	Yes	Yes	No

to leak ATP at 8 min after infection at the same rate as the infected PifA^+B^+ cell, and yet ribosomes are not immediately damaged, and protein synthesis continues for an additional 5 min.

Several matters need to be cleared up, therefore, before we will know whether or not the permeability hypothesis holds water. The results of Blumberg *et al.* may indicate that membrane damage is only a partial explanation for the abortive infection, or they may mean that leakage of ATP is an inadequate assay for the total amount of membrane damage sustained during infection of a wild-type male. Part of the difficulty in distinguishing between these two alternatives is that we do not know whether the Pif mutants really define two genes, or whether the PifA^-B^+ mutant simply contains a crippled Pif gene. In addition, it will help in evaluating the relative importance of ribosome damage and membrane damage when we know whether RNA synthesis is affected in the PifA^-B^+ male relative to the wild-type male, and whether or not ribosomes in the PifA^-B^+ male remain healthy even after the infection aborts.

A successful virus infection represents a very carefully balanced relationship between the virus and its host, which, in this case, has run amuck. There seems to be a T7 gene(s) which when expressed in male *E. coli* leads to permeability changes, and may ultimately be involved directly or indirectly in the observed paralysis of macromolecular synthesis and damage to ribosomes Especially because of the implied interaction with the cell envelope, it will be interesting to discover the normal role of this phage gene during T7 infection of female cells.

Hormone and antihormone action at the target cell

from Tom Blundell

A Dahlem Workshop on Antihormones and the Target Cell was held in Berlin on February 16–20, 1976. The proceedings will be published by Dahlem Konferenzen

SPECTACULAR advances in our knowledge have recently resulted from studies of hormone receptors. This is not only true for the membrane receptors of polypeptide hormones and catecholamines, but also for the intracellular receptors of steroid hormones, vitamin D metabolites and thyroid hormone. Can these new insights into the field of receptors now be useful in the design of biological tools and therapeutic agents?

This question was central to the Fourth Dahlem Workshop on "Hormone and Antihormone Action at the Target Cell" held in Berlin recently at which endocrinologists with a wide range of interests in hormone receptor interactions gathered together with biophysicists, molecular biologists and protein biochemists.

Unfamiliarity with the new no-lecture format of the Dahlem meetings resulted in a hesitant beginning but the discussions soon warmed up with the help of the generous hospitality and efficient organisation of Silke Bernhard and her colleagues. In the following days the debate ranged over a number of difficult and neglected areas in the study of hormone receptor interactions, and demonstrated the need for discussions between the various scientific disciplines represented.

The term "antihormone" refers to all those substances that act to attenuate hormone-induced responses regardless of their mechanism. Thus mechanisms changing the affinity of the receptor for the hormone by direct competitive binding of antagonists or indeed by allosteric effectors are included, as well as factors which affect hormone availability at the target cell, loss of receptors, and regulation of receptor synthesis.

For steroid receptors a combined steric and allosteric model presented by M Sherman (University of California Medical Center) seemed to be very useful In this model, a dimeric receptor can exist in two conformational states, one of which is inactive because the receptor molecule cannot enter the nuclei or cannot bind to the proper genomic sites or is not released normally from the nuclei. A steroid that binds the inactive state is an antagonist, and one that binds the active state is an agonist. Any steroid that exhibits some affinity for both states is a partial agonist because under some conditions it will be a suboptimal inducer of the function studied, but in the presence of a pure agonist it may act as a partial antagonist. Thus the antagonism by progesterone of glucocorticoid-induced tyrosine aminotransferase in hepatoma cells results from the formation of a glucocorticoid receptor–progesterone complex which does not undergo translocation to the nucleus. On the other hand the ability of steroids to act as partial agonists is exemplified by the partial action of 11-deoxycortisol on sodium transport. In this system aldosterone binds exclusively to the active state while the 11-deoxycortisol binds about half as well to the active and inactive states.

In the field of membrane receptors models seemed to be less in fashion and there was general emphasis on the need to define the macromolecular components more precisely. No hormone receptor or eukaryotic adenylate cyclase has as yet been purified although a characterisation using appropriate detergents as solubilising agents has been achieved for a number of membrane bound proteins such as Ca^{2+}–ATPase, glycophorin and the acetylcholine receptor. C. Tanford (Duke University Medical Center) emphasised the need for using detergents such as Tween 80 with long hydrophobic tails which simulate the membrane bilayer but pointed out that a rather different detergent, for example deoxycholate, may be preferable for initial disruption and fragmentation of the membrane. The presence of detergent bound to a protein receptor does not interfere with the determination of molecular weight using sedimentation equilibrium. With pure preparations, the conformational state of occupied and unoccupied receptors can be defined and the design of antihormones carried out on a more rational basis.

Many hormones have not evolved to fit best the receptor of the same species. Although there is good species specificity with vasopressin analogues, salmon calcitonin has high activity in man when compared with human calcitonin. In a similar way insulin receptors from different species bind insulins from various animals with similar relative affinities. J. Roth (National Institutes of Health, Bethesda) suggested that the receptor proteins may be under strong evolutionary constraints because they have other functions essential to the membrane. Thus similar molecules may exist in the membranes of cells to which the hormone has no biological response and even in prokaryotic cells. This would provide an explanation for the reported activity of insulin and glucagon on bacteria and algae. It also suggests that it may be worthwhile to explore the possibility of synthesising molecules which are better agonists than the natural hormone.

Another observation that gave rise to much discussion was the recent report on the enkephalin peptides which appear to be the natural opiates. These will clearly stimulate the search for other natural peptides which bind drug receptors. Conversely it implies that a non-peptide molecule such as morphine can bind a peptide receptor, and that in the design of therapeutic agents which are peptide-hormone agonists or antagonists it should be possible to use analogues of a very different chemistry. ☐

articles

Geological and palaeontological background of Hadar hominid site, Afar, Ethiopia

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The hominid-bearing Plio–Pleistocene sequence of the west central Afar sedimentary basin in Hadar contains 120–140 m of fossiliferous lacustrine and lake margin sediments. The Hadar sequence is described and divided into four members. The mammalian fauna indicates an age of ~ 3.0 Myr which is confirmed under the Hadar formation by K–Ar determinations on a basalt.

THE Afar is a depression at the northern end of the East African rift at the triple intersection of the trends of the East African rift, the Red Sea and the Gulf of Aden (Fig. 1). After three field seasons by the International Afar Research Expedition (IARE), the site of Hadar in the Afar promises to become one of the richest fossiliferous Plio–Pleistocene localities associated with the East African rift system. Here we present the generalised geology and palaeontology of Hadar.

Earth Resources Technology Satellite (ERTS) photographs of the Afar reveal a region of predominantly dark tone, marked with lineaments and volcanoes. The rocks are mostly fissure erupted flood basalt, divided into a few long north trending grabens and horsts, with rhyolitic volcanic centres. Aside from the Tendaho graben containing Lake Abbe, the only sediment-filled area is the west central Afar sedimentary basin composed of Plio–Pleistocene deposits adjacent to the Western Ethiopian plateau[1] (Fig. 1). East of the sedimentary basin the flood basalt constitutes three fault blocks. Running from north to south these are: the Mille block, comprising the Guralle acidic centre overlain by the Amado sequence of the west central Afar sedimentary basin; the Magenta block; and the tilted Galalu block interbedded at its top with the sediments seen south of Hadar, and referred to as the Rougous sedimentary sequence. K–Ar ages have been determined regionally for these basalts[2,3], with most of them between 2–4 Myr.

The Plio–Pleistocene sedimentary basin is ~ 60 km wide and 150 km long. Much of this area is capped unconformably by an uppermost unit of Pleistocene gravels and some bedded sands containing Acheulean artefacts[4]. The underlying Plio–Pleistocene strata have already been referred to as the Central Afar Group[5]. The IARE has studied sequences of this group regionally at Amado, Gewane, Gueraru, Hadar, Haouna, Leadu, Ledi, and Meschelle (Fig. 1). Fauna from these sites were tentatively referred to a time range of 2–4 Myr in comparison with other eastern African sites[5]. Thus, the sedimentary basin was contemporaneous with the volcanism just to the east.

The Hadar Formation

WE refer to the area of Plio–Pleistocene sediments exposed in the drainage of the Awash basin in the tributaries of Gona, Sidi Hakoma, Kada Hadar, Ounda Hadar and south of the Awash River (Fig. 2) as the Hadar site of the west central Afar sedimentary basin. Operationally we refer to the exposed sequence in this area as the Hadar Formation, even though the upper and lower contacts of the formation have not yet been fully explored. The sediments represent lacustrine, lake margin and associated fluvial deposits, related to a Plio–Pleistocene lake which may have occupied nearly the entire sedimentary basin, at least periodically. The offshore deposits are predominately clay sediments with the remains of fish and ostracods. Nearer shore deposits are primarily silts or calcareous mudstones; shore deposits are sands with shallow cross-bedding. Fluvial deposits are represented by pebbly gravels and sands with steep cross-bedded channel fills cut in underlying strata. For the Hadar site generally, the sequence consists of a higher percentage of fluvial facies to the west near the Gona tributary, and offshore facies to the east near the Ounda Hadar, compared with Kada Hadar where the largest percentage of nearshore facies occurs. From Sidi Hakoma to Kada Hadar (Fig. 2), where the members of the formation have been defined, the sequence has ~ 45% clays, 25% silts, 25% sands and sandstone and 5% volcanic, carbonate and biogenic deposits. Most of the vertebrate fauna is found in the sands and sandstones. The frequent occurrence of complete fossil specimens as well as partially associated skeletons, and the general lack of taphic abrasion indicates a low energy depositional environment and a possible high rate of sedimentation.

The formation has an exposed thickness of 120–140 m in the Kada Hadar tributary. The sequence dips northwards by 2–4° with just a few steep normal faults trending 5°N–20°E with displacements of 5–40 m (Fig. 2). Several minor erosional unconformities can be seen in the Hadar Formation, but no major time gaps are suggested. The Hadar Formation has been conveniently subdivided into four members, separated by major tuff horizons as shown in the geological column (Fig. 3). A geological map (Fig. 2) and a generalised cross section (Fig. 4) are presented here. Each member in this tributary has three or

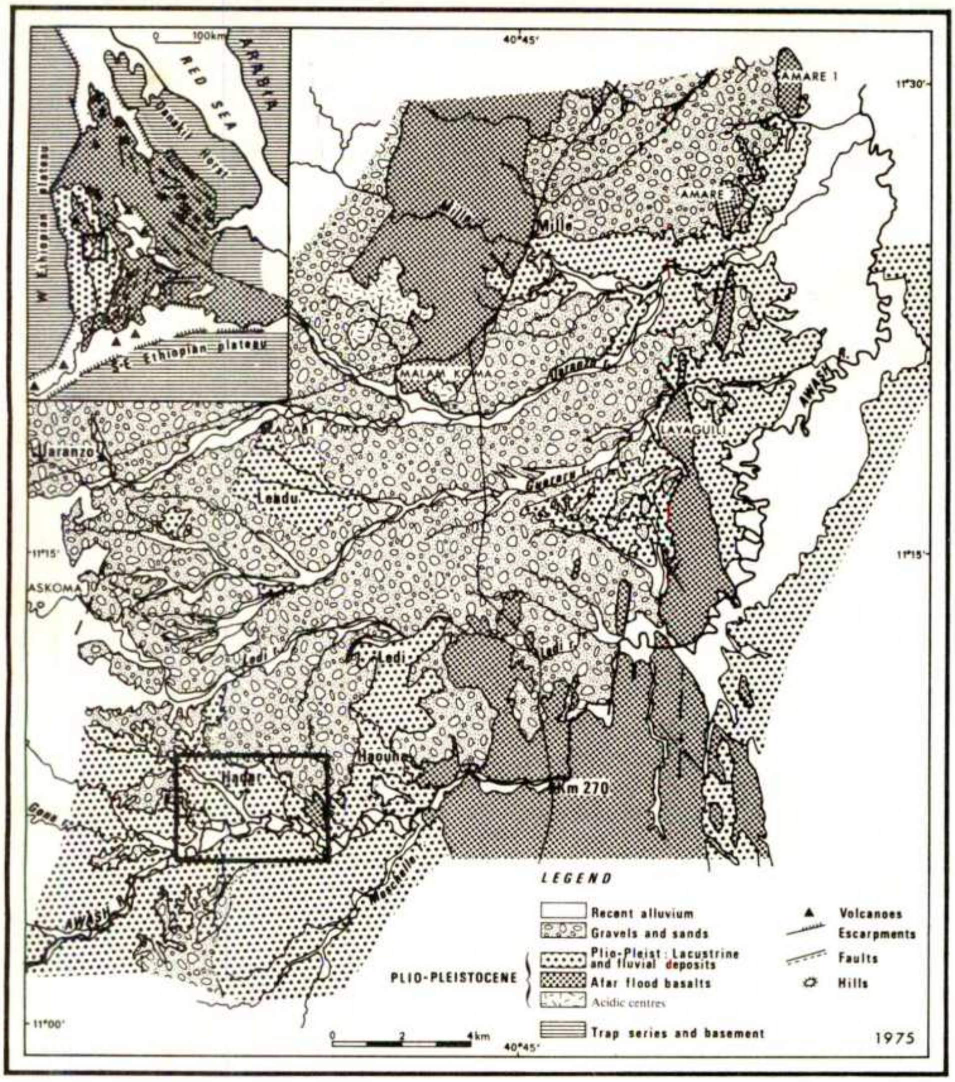


Fig. 1 General geological map of the north-western part of the west central Afar sedimentary basin (mapping with the collaboration of N. Page).

more submembers of alternating sands and clay–silt sediments. From the base to the top the members are: (1) the basal member (BM), 12 m exposed; (2) the Sidi Hakoma member (SH), with a series of white tuffs (SHT) at its base, 55 m thick; (3) the Denen Dora member (DD) with a series of thin tuffs (TT) in an extensive clayey marker horizon at its base, 25 m thick; and (4) the Kada Hadar member (KH), with a single tuff (KHT) at its base, 48 m thick.

Volcanics

In general the tuff horizons are laterally extensive and are nearly everywhere altered to a chalky white fine material. SHT and KHT have, however, been traced to palaeochannel fills in the underlying strata, where they are grey with a somewhat vitreous lustre, and consist of 95–98% glass shards derived from explosive pumice eruptions. Only the SHT channel fill shows closely spaced, steeply dipping cross bed sets in cut-and-fill troughs indicating a fast flowing current in the channel. The occurrence of fresh unaltered glass in the palaeochannels may arise from sorting of the coarsest glass, which resisted alteration.

At the eastern end of the Hadar site the Kadada Moumou subplateau is upheld by a single 1–4 m thick basalt flow, 60 m above the lowest exposures. The flow thins to the north-west where it ends. It lies over a section of largely clayey and silty sediments with two gastropod-rich layers (Fig. 4). Unconformably above the basalt is the post-Hadar Pleistocene gravel unit. In one locality, sediments of the Hadar Formation have been observed overlying the basalt. The Kadada Moumou subplateau geomorphic feature originated before the deposition of the overlying gravels when erosion was greatly slowed by exposure of the very resistant basalt. Extending from the north-western edge of the basalt into the dipping Hadar sedimentary sequence is a local unconformity with a relief of 4–6 m. Above the unconformity are local steep bedded sands with numerous boulders of the basalt. These sands contain abundant Hadar Formation fauna and are overlain by a distinctive sand unit which is laterally extensive within the Sidi Hakoma member. There is no fault between the Kadada Moumou flow and the sedimentary sequence. Twenty metres stratigraphically above the basalt level in the sediments is the persistent horizon (TT) which marks the base of the DD member. The basalt is thus in the upper portion of the Sidi Hakoma member.

The basalt is vesicular at the top, and denser, with few vesicles in the centre portion, and has calcite-filled vertical tubes near the base. The underlying clayey sediment exhibits a prismatic structure at the contact from baking. We cannot yet evaluate whether or not the flow is subaquatic.

Palaeontology

Over 6,000 specimens representing some 40 species of mammals including hominids[6,7] have been collected from > 250 localities in the Hadar area. They display remarkable preservation due to the unique taphonomic setting noted above. The collection can be closely compared to the Omo 1 Zone[8,9] (Usno Formation and the Basal Member and Members A and B of the Shungura Formation). Currently this time span in the Omo sequence is dated at ~ 2.6–3.1 Myr (ref. 10). The most significant elements of the assemblage for biostratigraphical correlation include: *Primelephas* (a progressive species with affinities to

Fig. 2 Geological map of northern Hadar.

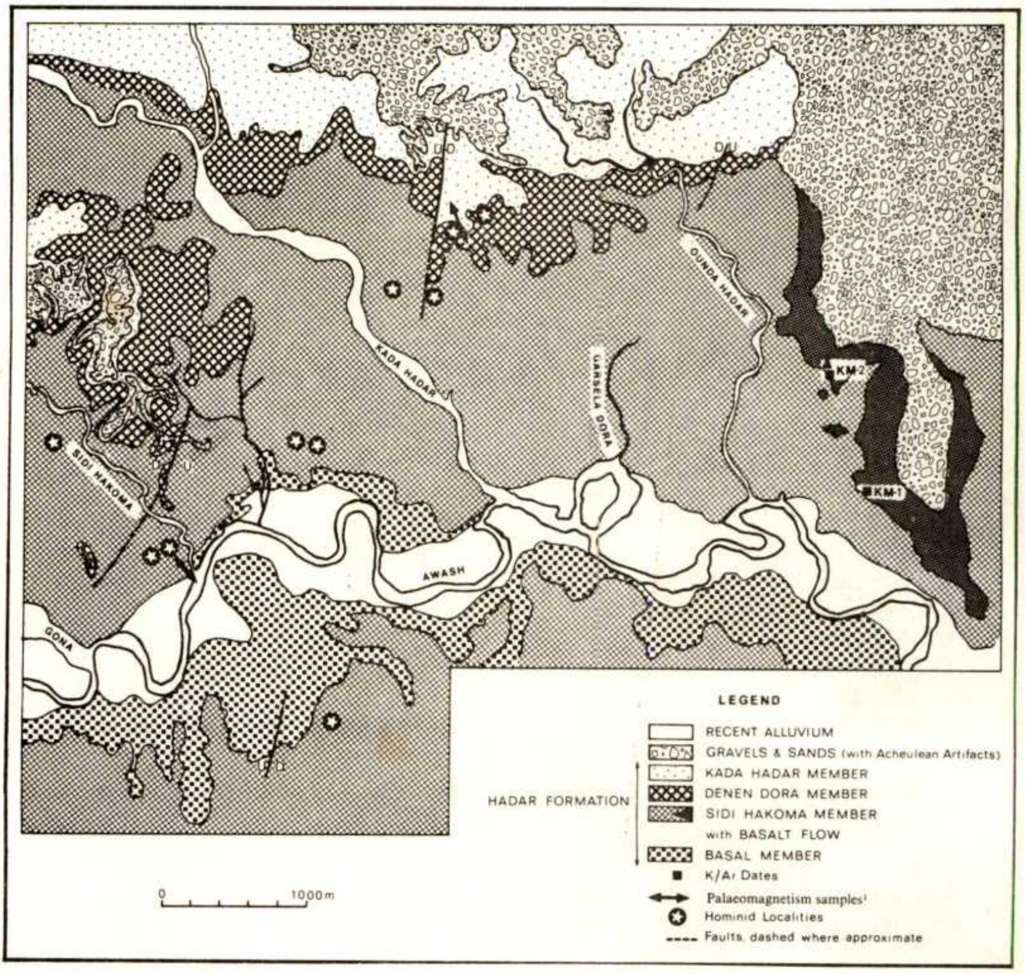
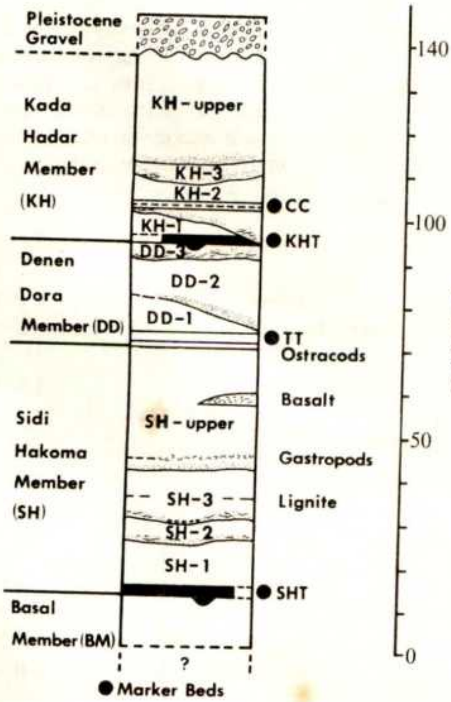


Fig. 3 Geological column of the Hadar Formation.



P. korotorensis from Chad), *Nyanzachoerus pattersoni*, *Notochoerus euilus*, *Loxodonta adaurora*, *Elephas recki* (stages 1 and 2 of Maglio's evolutionary scheme[11]), *Hipparion* cf. *primigenium* and *Hippopotamus* cf. *imaguncultus*. Table 1 presents a provisional faunal list for the Hadar site.

B. T. Gray has suggested a subdivision into three faunal

Fig. 4 Generalised geological cross section of the Hadar Formation. In the east, near Kadada Moumou the upper units have been extended over the basalt for stratigraphic clarity, but virtually all of these have been removed by erosion that predated the Pleistocene gravel.

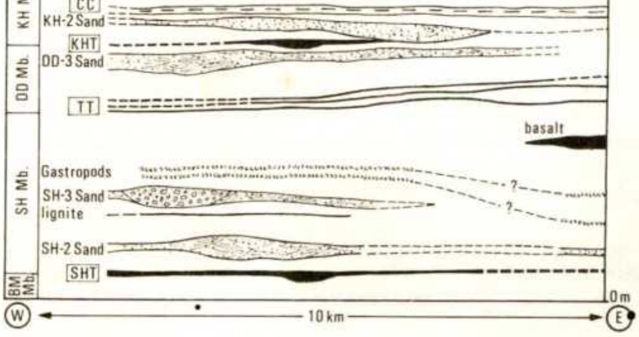


Table 1 Provisional faunal list from Hadar Central Afar

Mammalia
Artiodactyla
Hippopotamidae
Hippopotamus sp., *H.* cf. *imagunculus*
Hippopotamus sp.
Suidae
Sus sp.
Notochoerus sp., *N.* cf. *euilus*
Nyanzachoerus pattersoni
Giraffidae
Sivatherium maurusium
Giraffa sp., *G.* cf. *jumae*
Giraffa sp. nov.
Bovidae
Ugandax sp.
Tragelaphus sp., *T.* cf. *nakuae*
Tragelaphini, gen. et sp. indet.
Hippotragus sp.
Kobus sp.
Aepyceros sp.
aff. *Parmularis* sp.
Alcelaphini, gen. et sp. indet.
Neotragini/Cephalophini, gen. et sp. indet.
Ovibovini, gen. et sp. indet.
Perissodactyla
Equidae
Hipparion sp., *H.* cf. *primigenium*
Hipparion sp.
Rhinoceratidae
Ceratotherium sp., *C.* cf. *praecox*
Diceros sp., *D.* cf. *bircornis*
Proboscidea
Elephantidae
Elephas recki
?*Elephas* sp.
Loxodonta sp. *L.* cf. *adaurora*
Primelephas sp.
Deinotheroidea
Deinotheridae
Deinotherium bozasi
Rodentia
Hystrix sp.
Mastomys sp.
Xenohystrix sp., *X.* cf. *crassidens*
Xerus sp.
Tachyoryctes sp.
Pelomys sp.
Oenomys sp.
Tatera sp.
Carnivora
Canidae
gen. et sp. indet.
Hyaenidae
Crocuta sp.
Mustelidae
?*Enhydriodon* sp.
gen. et sp. indet.
Felidae
Megantereon sp.
?*Dinofelis* sp.
Primates
Cercopithecidae
Papio sp.
Theropithecus sp.
Colobus sp.
Hominidae
Australopithecus sp., *A.* aff. *robustus*
Australopithecus sp., *A.* aff. *africanus*
Homo sp.
Chiroptera
gen. et sp. indet.
Aves
gen. et sp. indet.
gen. et sp. indet.
Reptilia
Chelonia
Geochelone sp.
Trionyx sp.
Crocodilia
Crocodilus sp.
Ophidia
gen. et sp. indet.
Lacertilia
gen. et sp. indet.
Osteichthyes
Siluridae
gen. et sp. indet.
Mollusca
Gastropoda
gen. et sp. indet.
Bivalvia
gen. et sp. indet.
Arthropoda
Ostracoda
gen. et sp. indet.
Decapoda
Potamidae
gen. et sp. indet.

units: lower, middle and upper. The lower unit corresponds to SH-1 and SH-2 submembers, the middle to SH-3 through DD-2, and the upper to DD-3 and above. The vast majority of the middle unit fauna is derived from DD-1 and DD-2. Most taxa are represented in all three units, although proportions do show some interesting variations. The lower unit is characterised by the association of Elephantidae, *Notochoerus euilus*, *Nyanzachoerus pattersoni*, *Tragelaphus* cf. *nakuae*, *Aepyceros* sp., and cercopithecines. The most common elements of the middle unit are *N. euilus*, *T.* cf. *nakuae*, *Aepyceros* sp., and abundant *Kobus*. Elephantidae are less well represented. The upper unit contains *N. euilus*, *T.* cf. *nakuae*, *Kobus* sp., Alcelaphini, *H.* cf. *primigenium* and Elephantidae.

After more detailed study the subdivision will provide valuable palaeoecological and chronological data. The surprisingly great number of *Kobus* specimens in the middle unit, for instance, or the absence of *Nyanzachoerus* in the upper unit is interesting. Clearly, however, several environmental zones are being sampled in each of these units, and further subdivision is required before detailed interpretations may be proffered.

Geochronological and palaeomagnetic evidence

One of us (J.L.A.) is performing a detailed geochronological study of the Hadar site. Thus far only five K–Ar measurements are available, two for the Kadada Moumou basalt and three for glass shard samples from the SHT channel fill (Fig. 2). The analyses were performed in an extraction system with an ^{38}Ar spike pipette and an MS-10 mass spectrometer with little memory and no mass 36 background. Table 2 presents the analytical data for these samples. Because of the high percentage of atmospheric argon, these determinations are, as yet, relatively imprecise. Two basalt determinations from separate localities in the flow are analytically consistent at 2.95±0.2 Myr and 3.0±0.2 Myr. Both samples consist of fine 0.015 mm plagioclase and augite and minor olivine in a magnetite-rich cryptocrystalline matrix, which comprises 35% of the rock. The content includes 6% of calcite fillings about 0.2 mm in size, surrounded by a fine yellowish, interstitial material (a possible alteration of glass) equalling 1% of the rock's content. The results of the glass shard separates represent three different experimental treatments and are inconsistent, ranging from 3.1–5.3 Myr, each with an analytical uncertainty of 0.3 Myr.

Table 2 Analytical results of K–Ar dating

Sample	^{40}Ar* g^{-1}	^{40}Ar*/^{40}Ar × 100	%K$_2$O†	K–Ar age (Myr‡)
A Basalt				
KM-1-73	0.0291	20.6	0.654±0.004(2)	3.0±0.2
KM-2-74	0.0327	15.2	0.748±0.004(4)	2.9_5±0.2
B SHT shard§				
$4l_1$	0.092	9.0	1.995±0.015(2)	3.1±0.3
$4l_2$	0.121	12.3	1.97	4.1±0.3
$4l_3$	0.157	16.6	1.98	5.3±0.3
LP6 40/60 Biotite standard (ref. 1)	18.14±0.30(5)		10.10±0.16(10)	

*Radiogenic argon, 10^{-10} mol.

†Average and average deviation, number of measurements in parentheses.

‡^{40}K/total K = 1.19×10^{-4}; λ_b = 4.72×10^{-10} yr^{-1}; λ_e = 5.85×10^{-11} yr^{-1}; age uncertainty calculated using 0.3% uncertainty in isotopic composition of sample and atmospheric argon, a 1% uncertainty in argon spike, 1% uncertainty in K$_2$O, and 1% uncertainty in sample homogeneity.

§Index of refraction = 1.500. All samples 150/200 mesh slide concentrates of flakey bubble sides, $4l_2$ and $4l_3$ further refined by floating in ρ = 2.52 g cm^{-3} liquid; $4l_1$ prebaked overnight in vacuum at 250 °C, $4l_2$ and $4l_3$ at 175 °C; $4l_3$ treated 13 min with cold 3% HF.

Until further experiments, these inconsistent results are not understood. They may result from the presence of excess radiogenic argon in the glass. The thicker glass shards, which represent interstices between three or more bubbles, display a microbubble structure which could house such magmatic ^{40}Ar.

Figure 2 shows the location of two sections sampled for preliminary palaeomagnetic examination, in addition to 3 samples of the Kadada Moumou basalt (measurements made by T. J. Schmitt). The lower palaeomagnetic sequence, consisting of 15 samples, spanning 25 m, derives from the basal member up to the SH-2 unit. The upper section comprises 10 samples spanning 20 m of the lower KH member (KH-1, 2 and the lower part of 3). Both sedimentary sequences are magnetised normally, whereas those of the basalt are reversed. All determinations retain their orientations under a.c. demagnetisation. These palaeomagnetic data are consistent with the basalt date, if the reversal is interpreted as representing the Mammoth or Kaena events within the Gauss normal epoch, itself represented by the two normally polarised sedimentary sequences above and below the basalt. This interpretation is to be tested by future palaeomagnetic sample collections from the intervening sedimentary sequence which, to be consistent, should contain the reversal recorded by the basalt.

The Hadar sequence will be a reference from which stratigraphic and palaeontological studies may be extended into adjacent parts of the west central Afar sedimentary basin. The palaeontology, geochronology, and palaeomagnetism tentatively suggest a short duration of sedimentation for the formation, probably less than a million years with no major time gaps. Thus the Hadar Formation together with its rich fauna and varied geological environments, will permit precise palaeoecological and palaeogeographical reconstruction for an important and previously poorly known segment of later Cainozoic hominid evolution.

Support from the following is acknowledged: the CNRS, the NSF, the Cleveland Museum of Natural History, the Wenner-Gren Foundation for Anthropological Research and Ethiopian Airlines. Special gratitude is expressed to the Ethiopian Government and the Ministry of Culture and Youth. Of great logistical support to the expedition have been the efforts of Richard Wilding, Dr Robert Caldwell and Willard Brown.

Received September 25, 1975; accepted February 9, 1976.

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Plio–Pleistocene hominid discoveries in Hadar, Ethiopia

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The International Afar Research Expedition has now recovered remains of twelve hominid individuals from geological deposits estimated to be ~ 3.0 Myr in Hadar, Ethiopia. A partial skeleton represents the most complete hominid known from this period. The collection suggests that Homo *and* Australopithecus *coexisted as early as 3.0 Myr ago.*

FOLLOWING a short reconnaissance expedition in 1972 (ref. 1) to the central Afar, one of us (M.T.) organised the International Afar Research Expedition. We have now codirected two field campaigns (September–December, 1973 and 1974 (refs 2 and 3)) in the area known as Hadar (see Fig. 1).

Although a number of other excellent fossil sites are known from the central Afar[4], Hadar was selected for intensive exploration for a number of reasons: first, the fossil vertebrate assemblage suggested a considerable antiquity (~ 3–4 Myr); second, the area is extremely rich in splendidly preserved fossils, deposited in low energy environments; third, the deposits are heavily dissected and characterised by clear marker-horizons which are laterally continuous; and fourth, the thick series of lake sediments contains a number of volcanic horizons available for absolute radiometric age determinations.

The Hadar site is located in the Afar depression in the west central Afar sedimentary basin[5] at 11°N and 40°30′E. It now encompasses ~ 42 km^2, but extension of study into adjacent, previously unexplored regions will substantially increase the area.

Preliminary palaeontological investigations, particularly of the suids and the elephants, have suggested a biostratigraphic correlation of the Hadar Formation with the Usno and the lower portion of the Shungura Formations[5,6]. Two radiometric K–Ar age determinations for a basalt support this with an age estimate of 3.0 ± 0.2 Myr (ref. 5).

All hominid fossils (Table 1), and most of the vertebrate material, were surface finds, although some small scale excavation and sieving operations were undertaken. A painted marker was placed at each palaeontological locality, each located on a map and the geological section carefully examined to determine the horizon yielding fossil material (see Fig. 2). At each hominid locality, a topographical map and detailed stratigraphical sections were drawn. Samples of matrix adhering to fossil hominid specimens are undergoing mineralogical and sedimentological analyses for comparison with sediment samples taken at their collection points. Their completeness and lack of anataxic abrasion[7] virtually precludes the possibility that the specimens were moved over great distances.

1973 hominid discoveries

The first discovery of fossil hominid remains in the Hadar region occurred on October 30, 1973, when four associated leg bone fragments (AL 128, 129) were collected from a mudstone horizon. These consist of fragmentary right and left proximal femora associated with a right proximal tibia

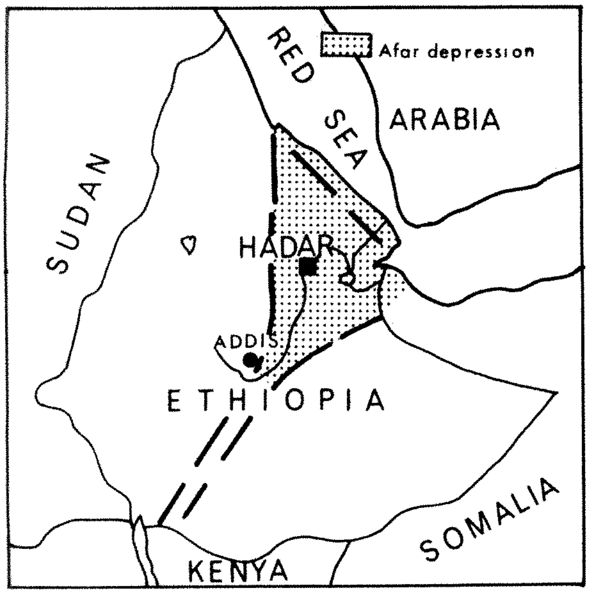

Fig. 1 Location of Hadar.

and distal femur. Their proximity to one another, as well as the morphological and size similarities of the proximal femora, strongly suggest that they represent a single individual. This provides a complete knee which will greatly enhance our understanding of the biomechanics of this important joint in early hominids (see Fig. 3).

Both proximal femora lack heads and necks. The left is best preserved with the shaft broken ~ 38.0 mm below the lesser trochanter. From the remaining portions of the femora it is clear that the neck is flattened anteroposteriorly and that its cross section is oval. There is an indication of trochanteric flare, the trochanteric fossa is well marked, a quadrate tubercle is clearly discernible, muscle markings are prominent on the greater trochanter, the spiral line is pronounced and the lesser trochanter is visible from the anterior aspect. Both fragments, particularly the right, exhibit some degree of predepositional crushing, which is suggestive of carnivore activity.

Table 1 Fossil hominid specimens from Hadar

Afar locality (AL)	Date of discovery	Description
	1973	
128–1	30 October	Left proximal femur fragment
129–1a	30 October	Right distal femur
129–1b	30 October	Right proximal tibia
129–1c	30 October	Right proximal femur fragment
166–9	11 December	Left temporal fragment
	1974	
188–1	16 October	Right mandibular corpus; M_2–M_3
198–1	18 October	Left mandibular corpus; C–P_4, dm, M_1–M_2
198–17a,b	5 December	Left I^1 and I^2
198–18	5 December	Right I_2
199–1	17 October	Right maxilla; C–M^3
200–1a,b	17 October	Complete maxilla; 16 teeth. Right M_1
211–1	20 October	Right proximal femur fragment
228–1	27 October	Diaphysis of right femur
241–14	22 December	Left lower molar
266–1	16 November	Mandibular corpus; left P_3–M_1, right P_3–M_3
277–1	19 November	Left mandibular corpus; C–M_2
288–1	24 November	Partial skeleton: occipital and parietal fragments; mandibular corpus with left P_3, M_3, right P_3–M_3, mandibular condyles; right scapula fragment; right humerus; proximal and distal left humerus; proximal and distal right and left ulnae; proximal and distal right radius; distal left radius; left capitate; 2 phalanges; 6 thoracic vertebrae and fragments; 1 lumbar vertebra; sacrum; left innominate; left femur; proximal and distal right tibia; right talus right distal fibula; numerous rib fragments.

The distal femur is undistorted, and both condyles are complete and intact. It is small, with the lateral condyle measuring 39.0 mm anteroposteriorly and 18.9 mm mediolaterally. This fragment demonstrates a number of anatomical details which are intimately related to bipedal locomotion[8,9]: the bicondylar angle is rather high, the lateral lip of the patellar groove is raised and the lateral condyle flattened and elongated.

The associated proximal tibia is intact except for some slight abrasion around the periphery of the superior articular surface. It also is small, the total preserved articular surface measuring 50.7 mm mediolaterally and 33.0 mm anteroposteriorly. The tibial tuberosity is pronounced and is limited proximally by the transverse groove; the soleal line is distinct on the posterior surface of the shaft and the interosseous membrane attachment is indicated by a roughened line on the medial aspect of the shaft; the head is slightly retroflexed with only minor tibial torsion; and the intercondyloid eminence is prominent with well developed intercondyloid fossae.

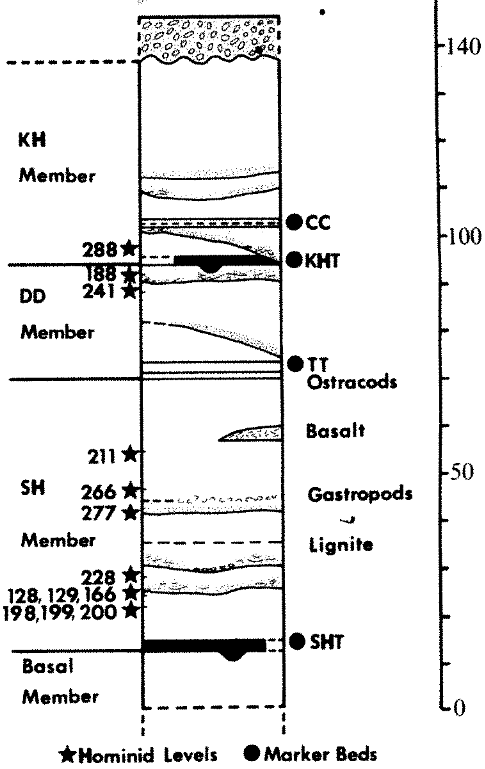

Fig. 2 Stratigraphic positions of Hominid Horizons. ★, Hominid levels; ●, marker beds.

A heavily eroded temporal fragment (AL 166–9) constitutes the only other hominid discovery from the 1973 field season. The specimen derives from a sandstone horizon a few metres stratigraphically above the level of the postcranial material. The temporal has been extensively eroded, with most of the petrous portion broken away, and extensive pneumatisation is exposed in the mastoid and zygomatic regions. The mandibular fossa is broad and flat, bordered by a postglenoid process, but open anteriorly with only a slight articular tubercle. There is a prominent entoglenoid process on the medial wall which is broken exposing a large pneumatisation. The mastoid is large and globular. The external auditory meatus is oval in section with a thick tympanic plate.

1974 hominid discoveries

Our sample of the Hominidae has been greatly augmented with additional field work. The 1974 investigations yielded

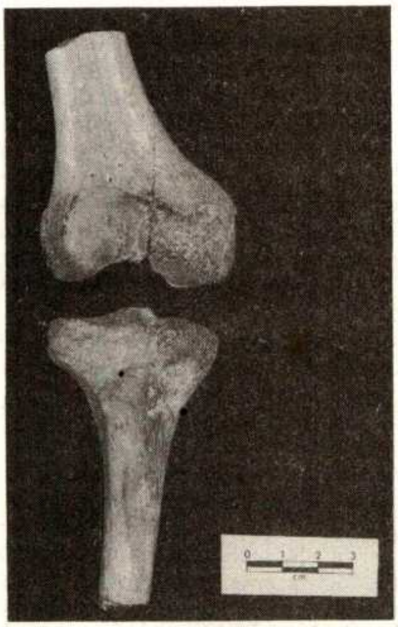

Fig. 3 Anterior view of AL 129–1a and 1c

remains of 10 additional individuals represented by dental, cranial and postcranial elements, including a remarkably intact partial skeleton.

Stratigraphically just below the 1973 hominid discoveries, one complete (AL 200–1) and one half maxilla (AL 199–1) (see Fig. 4) were recovered by Ato Alemayehu Asfaw within 15 m of one another and from the same mudstone horizon.

The AL 200 specimen is an undistorted palate with full dentition and an associated right M_1. The general pattern of dental wear is interesting, in that the incisors exhibit extensive 'ribbon like' wear and dentine exposure relative to the postcanine teeth. In addition, the canines and the P^3s have suffered antemortem enamel chippage on their buccal surfaces during the specimen's lifetime. The tooth rows of AL 200 are subparallel and the arch is relatively long. The anterior portion of the arch is broad to accommodate the large central incisors and marked diastemata occur between the lateral incisors and the canines. The palate is shallow, becoming somewhat deeper posteriorly.

In lateral view this specimen exhibits pronounced alveolar prognathism. The zygomatic root is situated above the first molar, the maxillary sinuses are large and the lower nasal margin is guttered.

The right half maxilla is smaller in size and is less complete, with C–M^3, as well as both incisor sockets and the I^2 root. The dentition is smaller than that of AL 200 (Table 2), but the similarity in morphological detail is striking. Although the incisors are lacking, it is apparent from the root sockets that the anterior portion of the arch was broad and somewhat squared-off as in AL 200. The specimen is broken close to the midline exposing the incisive canal. The palate is shallow and the greater palatine foramen is present. The inferior nasal margin is not sharp and in lateral view alveolar prognathism is well developed. The maxillary sinus is large and the zygomatic root is situated above the distal portion of the first molar.

A partial mandible (AL 266–1) was located in a horizon of sandy clay, ~ 10 m stratigraphically below the basalt flow[5]. This specimen includes the complete right corpus with P_3–M_3, the symphysis with canine and incisor roots, and a portion of the left corpus with P_3–M_1 (Table 3). The dentition is moderately worn with the third molar having just come into occlusion and showing only minor wear facets. The anterior portion of the dental arch is rounded and the dental rows are straight and slightly divergent posteriorly. The post-incisive planum is only moderately developed and the corpus in the region of M_1 is 21.7 mm thick and 31.0 mm deep.

A right mandibular fragment (AL 188–1) containing M_2–M_3 and roots of P_3–M_1, was collected from a sandstone horizon immediately below KHT. The molars are heavily worn. The mandible exhibits some wind abrasion, but it is possible to estimate a thickness of 20.7 mm and a depth of ~ 32.0 mm in the region of M_1.

AL 277–1, a fragmentary left mandible containing C–M_2 and sockets for I_1–I_2, is derived from a sandstone horizon. It is broken just to the right of the midline and distal to M_2. The occlusal wear is heavy with the canine worn flat. Anteriorly the fragment gives the impression of being deep, measuring ~ 41.0 mm just distal to P_3. A prominent post-incisive planum is present, as well as a slight inferior mandibular torus. The inferior surface of the symphysis is flattened and has distinct mental spines. The corpus exhibits slight eversion.

An interesting left half mandible (AL 198–1) was recovered from a mudstone horizon, stratigraphically equivalent to AL 199 and AL 200. The specimen is broken near the midline with the I_1 socket and the root for I_2 preserved. The C–P_4 and M_1–M_2 are present, with a retained deciduous molar located distal to the P_4. The deciduous nature of this tooth is confirmed by a radiograph demonstrating a widely divergent root system. Substantial occlusal wear is evident on P_4–M_2. The corpus is broken just distal to M_2, exposing a portion of the mesial root of M_3 immediately superior to the mandibular canal. The mandible is lightly built, being 16.0 mm thick and 31.9 mm deep at M_1.

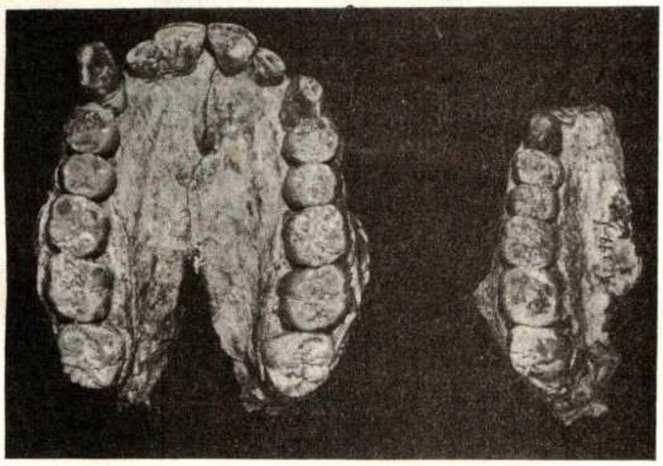

Fig. 4 Occlusal views of AL 200–1a and AL 199–1

Table 2 Dental measurements of AL 200–1a and AL 199–1 (mm)

	Left		Right	
AL 200–1a	Mesio-distal	Bucco-lingual	Mesio-distal	Bucco-lingual
I^1	10.8	8.3	10.9	8.5
I^2	7.4	7.1	7.3	7.0
C	9.4	10.9	9.4	11.0
P^3	9.0	12.2	8.9	12.2
P^4	8.5	12.2	8.5	12.1
M^1	11.8	13.1	11.8	13.2
M^2	13.8	14.8	13.7	15.0
M^3	14.2	15.0	14.3	15.0
AL 199–1				
C			8.7	9.3
P^3			7.3	11.2
P^4			7.1	11.2
M^1			10.1	12.0
M^2			11.7	13.5
M^3			11.3	(12.7)

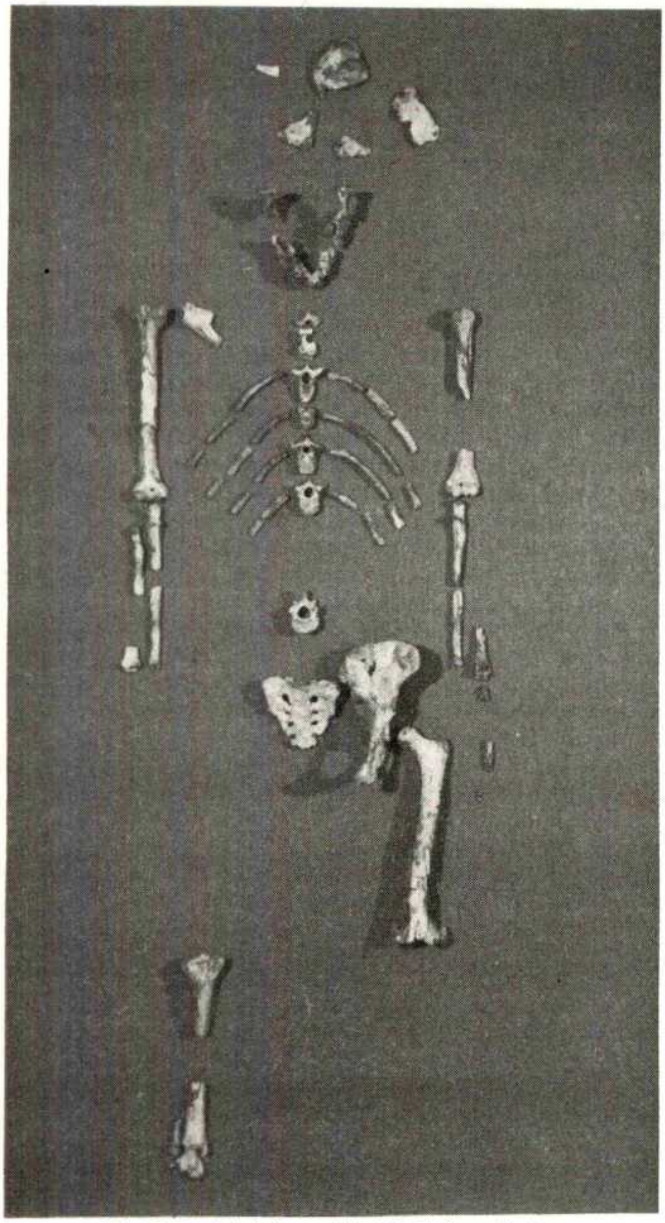

Fig. 5 Partial skeleton (AL 288–1) from Hadar

It exhibits some modelling and the inferior margin is thin and not bulbous.

During screening operations, three additional teeth were located at this locality: a right I_2 (AL 198–18), a left I^2 (AL 198–17a) and a left I^1 (AL 198–17b). The interproximal facets on the upper incisors match perfectly, suggesting that they are from the same individual. Because of the close association of these teeth with the AL 198 mandible, we tentatively assign them to the same individual.

Stratigraphically just below the basalt flow a fragmentary right proximal femur (AL 211–1) was collected. The specimen is somewhat abraded with the head and neck missing and most of the greater trochanter. The neck is very flattened, no trochanteric flare is present, the intertrochanteric line is weakly expressed, the spiral line is well developed and the lesser trochanter is not visible from the anterior aspect. The specimen is quite large and fairly robust.

AL 228–1 is a distal portion of a femoral diaphysis recovered from a sandstone. It is not large, but exhibits strong development of the linea aspera. It is smaller than AL 129–1A.

The partial skeleton

The discovery on November 24 of a partial skeleton (AL 288–1; see Fig. 5) eroding from sand represents the most outstanding hominid specimen collected during the 1974 field season. The stratigraphic horizon yielding the skeleton is situated just above the KHT, which has not yet been dated. Fossil preservation at this locality is excellent, remains of delicate items such as crocodile and turtle eggs and crab claws being found. It is obvious that this discovery provides us with a unique opportunity for reconstructing the anatomy of an early hominid in far more detail than has been previously possible. Extensive descriptive and comparative studies are projected for the AL 288 partial skeleton and will provide us with details of stature, limb proportions, articulations and biomechanical aspects. Three weeks were devoted to intensive collecting and screening to ensure the recovery of all bone fragments from the site. Laboratory preparation and analysis has only just begun, and in this report it is possible to mention only a few salient points.

The mandible is not heavily built; it is 30.0 mm deep and 19.0 mm thick in the region of M_1. The M_3s are fully erupted and occlusal wear facets are just appearing. The symphysis is intact with a slight post-incisive planum. Although the incisor crowns are absent, it is apparent that this region was quite small. The remaining dentition is small and not very worn. The P_3s are interesting with a sloping buccal surface and almost no development of a metaconid. The form of the dental arch as well as the body of the mandible is distinctly V-shaped.

The cranium is not sufficiently complete to estimate cranial capacity. The cranial bones are thin, exhibit no sutures (internally or externally) and no marked development of nuchal or temporal musculature is present.

The left innominate is complete, although it is somewhat distorted in the pubic region and particularly in the area of sacral articulation. In size the specimen resembles Sterkfontein (Sts) 14; the ilium, however, gives the appearance of being higher, and the anterior border is relatively straight. A strongly developed anterior inferior spine is apparent. The acetabulum is shallow when compared with modern man and with Sts 14. The sciatic notch is broad, the subpubic angle obtuse and the pubis exhibits a pronounced vental arc, all of which suggests the skeleton belonged to a female. When viewed from the superior aspect, the base of the sacrum is divided into thirds, with the diameter of the sacral body equal to each of the alae. This again suggests that AL 288 was female.

A complete left femur is associated with the innominate, but the distal portion is badly crushed. Its total length has been estimated at 280 mm but may be slightly revised when the distal end is reconstructed. The femur has minimal trochanteric flare, the neck is anteroposteriorly flattened, an intertrochanteric line is present and the lesser trochanter is not visible from the anterior aspect.

The proximal right tibia is nearly identical in size and morphology to AL 129–1b. The distal tibia is associated, and articulates with a talus and a distal fibula.

The right humerus is complete with some crushing of the proximal end. Its total length is estimated at ~ 235 mm giving a value of 83.9 for the humeral–femoral index. The distal end possesses a marked ridge separating the capitulum and trochlea.

Significance of the Hadar hominids

Detailed studies of the Hadar hominids have just recently been initiated and definitive interpretations are not yet possible. Because of the geological antiquity, and in some instances the completeness of the specimens, however, we proffer a number of preliminary impressions concerning their phyletic affinities and resemblances to other specimens.

The partial skeleton exhibits a number of similarities to the Sterkfontein sample. Specifically, the size of the pelvis and to some degree its morphology are reminiscent of Sts 14. The shallow acetabulum and relatively high ilium, how-

Table 3 Dental measurements of AL 266–1(mm)

	Left		Right	
AL 266–1	Mesio-distal	Bucco-lingual	Mesio-distal	Bucco-lingual
P_3	9.1	10.1	9.2	10.1
P_4	8.9	11.0	9.4	10.8
M_1	12.1	11.9	12.1	12.0
M_2			13.3	14.0
M_3			15.3	13.7

ever, demonstrate certain divergences from the Sterkfontein specimen and possibly reflect a somewhat more primitive status for the AL 288 specimen. The associated V-shaped mandible is noteworthy. Leakey[10] has drawn attention to mandibular shape and has suggested that KNM-ER 1482 (Kenya National Museum, East Rudolph), and Omo 18 should be considered primitive because of their V-shaped contours. Should this prove to be a diagnostic character, it is possible that AL 288 retains more primitive features than *Australopithecus africanus*, recognised from Sterkfontein.

Previously the 1973 postcranial material has not been assigned to a taxon. It is now clear that it should probably be included in the same category as the AL 288 specimen because of the striking similarity of the proximal tibial fragments in size and morphology as well as the preserved femoral fragments. This is important because: there is now evidence of at least two individuals of a very small hominid in Hadar, and the AL 128 and 129 specimens are situated stratigraphicaly 80 m below the partial skeleton (Fig. 2).

The presence of another taxon is suggested by two other specimens in the Hadar hominid collection. AL 211–1 resembles very closely Olduvai Hominid (OH) 20 (ref. 11) as well as the two proximal femora from Swartkrans (SK 82 and SK 97). Similarities are not only in size but also in morphological detail; stout shafts, flattened necks, a lack of trochanteric flare and posteriorly facing lesser trochanters not visible in anterior view.

The temporal fragment (AL 166–9) resembles material from Swartkrans and East Rudolf which has also been assigned to a large *Australopithecus* pattern, particularly in the large bulbous mastoid process and heavy pneumatisation, as well as the broad temporal shelf. It is, however, less typical of this pattern in the broad, flat mandibular fossa and absence of a strong articular tubercle.

The close association of AL 199–1 and AL 200–1, as well as their stratigraphic equivalence, is important because of the high probability that they sample the same taxon. Except for size differences, these two specimens are remarkably similar, and suggest variation within a single taxon. The complete maxilla has large canines, broad central incisors and large posterior dentition. These characters as well as other details suggest resemblances with some *Homo erectus* material, particularly *Pithecanthropus* IV. This dental pattern is also seen in KNM-ER 1590 from East Rudolf, a specimen with large cranial capacity assigned to the genus *Homo*[10,12]. It must be recognised that other aspects of the AL 200 maxilla are 'primitive', such as the guttered nasal margin and the alveolar prognathism.

The AL 266–1 and AL 277–1 mandibles resemble, in details of the dentition and mandibular morphology, other specimens assigned to *Homo*, such as OH 7 (ref. 13) and KNM-ER 1802 (ref. 10).

On the basis of the present hominid collection from Hadar it is tentatively suggested that some specimens show affinities with *A. robustus*, some with *A. africanus* (*sensu stricto*), and others with fossils previously referred to *Homo*.

The understanding of Plio–Pleistocene hominid remains is undergoing intensive revision and re-evaluaton, and we stress that our identifications and phylogenetic interpreta- such as the guttered nasal margin and the alveolar prog- liminary. With continued collection of specimens from the three million year-old time range in Hadar and elsewhere, and with additional detailed studies and comparisons, our attempts to interpret the earliest hominds should become more clear.

Special gratitude is expressed to Y. Coppens for his assistance in preparation of this manuscript. Owen Lovejoy also provided helpful comments.

Received September 25, 1975; accepted February 9, 1976.

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Sequence of promoter for coat protein gene of bacteriophage fd

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*The nucleotide sequence in the promoter region for the coat protein gene of phage fd has been determined. This sequence contains an endonuclease R·*Hha *cleavage site at the fifteenth nucleotide upstream from the RNA start site. Cleavage results in loss of promoter function. Comparison with the sequence of another fd promoter indicates that the longest sequence common to both was TATAAT in the region in which RNA polymerase forms a stable initiation complex.*

ELUCIDATION of the structure of DNA specifying the initiation of transcription is essential to understand the mechanism of gene expression. Although the sequences of several different promoters have been determined[1-7], the information still seems to be insufficient to define the structure essential for promoter function. In previous work in which the promoter regions were localised on the cleavage map of fd[8,9], two strong promoters were shown to be located on two separate fragments, named *HapC* and *Hap-HgaV*. The nature of these promoters was very similar: RNA polymerase binds tightly to these fragments in the presence of GTP and initiates G-start RNA with almost the same efficiency. The sequence in the promoter region on *Hap–HgaV* (named G2 promoter) has been determined[7]. In this report, the promoter region on *HapC* (named G3 promoter) was sequenced to find the sequence common to both promoter regions.

HapC contains the entire gene for the coat protein (B protein) of fd[10,11], and RNA of about 350 bases (G3 RNA) is efficiently synthesised on this fragment. This RNA contains the sequence for the coat protein (M.T. *et al.*, unpublished). Restriction endonuclease (R) *Hae*III cleaves *HapC* into two pieces: *HapC–Hae 1* and *HapC–Hae 2.* RNA of about 200 bases (G3′ RNA) is synthesised only on *HapC–Hae 1*. Comparison of the sequences of G3 and G3′ RNAs indicated that G3′ RNA was yielded by termination of G3 RNA at the R·*Hae*III cleavage site. *HapC–Hae 1* can be further cleaved by R·*Hha* into two pieces, *Hha 1* and *Hha 2*. No RNA was initiated, however, on the resulting fragments; the ability to form a stable complex with RNA polymerase was also destroyed by this cleavage. The positions of the cleavage sites and of the G3 RNA start site on *HapC* are indicated in Fig. 1. The sequences in the region preceding the G3 RNA start site and in the region in which RNA polymerase forms a stable initiation complex (RNA polymerase binding site) were determined by the following procedures.

(1) In low salt conditions, *HapC–Hae 1*-directed RNA synthesis was initiated at two additional sites, although RNA initiation at the G3 promoter was still predominant. Figure 2*a* and *b* shows autoradiographs of the low salt and high salt (0.15 M KCl) transcripts for comparison. The direction of transcription and the regions covered by these transcripts have been determined by hybridisation to fd phage (+ strand) DNA and each fragment of *HapC*. Since both the low salt transcripts, named (+)RNA·LS and (−)RNA·LS, covered the region preceding the G3 RNA start site (Fig. 1), these RNAs were further fractionated by hybridisation to each *Hha* fragment and by partial T1 RNase digestion, and the sequences of the resulting fragments were determined.

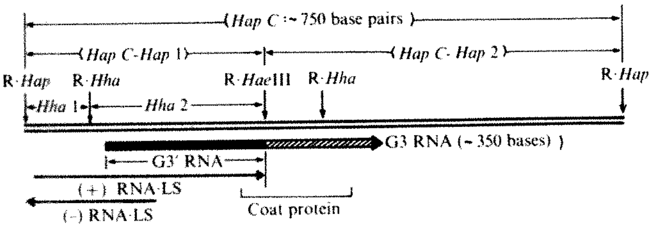

Fig. 1 Cleavage and transcription maps of the *HapC* region of fd DNA. Location of *HapC* on the cleavage map of fd DNA is given in ref. 8.

(2) *HapC–Hae 1* forms a stable initiation complex with RNA polymerase in the presence of GTP: the region protected by RNA polymerase was isolated after treatment with DNase I. In accordance with other observations[6,12–14], the isolated fragment was about 45 base pairs, as determined by gel electrophoresis. The polymerase-protected fragment was denatured by heating, and incubated in an RNA-synthesising system containing a primer oligonucleotide[12]. The resulting transcripts were fractionated by hybridisation to fd phage DNA and by gel electrophoresis, and their sequences determined.

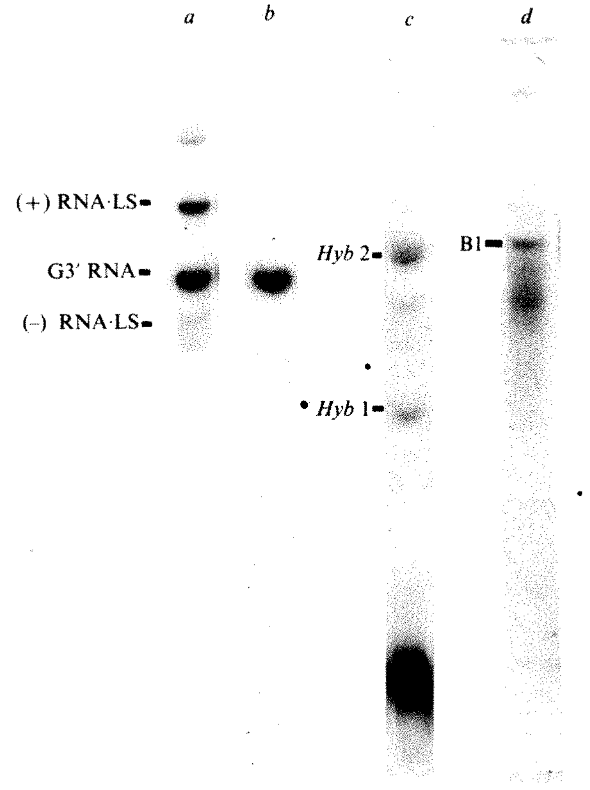

Fig. 2 Gel electrophoretic patterns of *a*, RNA formed on *HapC–Hae 1*, at low salt; *b*, RNA formed on *HapC–Hae 1* at high salt; *c*, portions of (+)RNA·LS hybridised to the R·*Hha*-digest of *HapC–Hae 1*, and *d*, RNA formed on the RNA polymerase-protected fragment. *a*, *HapC–Hae 1* (about 2 pmol) was incubated for 1 h at 37 °C in a reaction mixture (1 ml) containing 8 mM $MgCl_2$, 40 mM tris (pH 7.9), 0.1 mM dithiothreitol, 50 μM (α^{32}P)GTP and 0.2 mM each of three other NTPs, and about 15 pmol RNA polymerase. The transcripts were isolated and electrophoresed for 3 h at 150 V on a 5% polyacrylamide gel (0.6 cm × 12 cm) containing 7 M urea, as in ref. 12. *b*, RNA was synthesised on *HapC–Hae 1* and electrophoresed as in (*a*), except that 0.15 M KCl was added to the reaction mixture. *c*, RNA was extracted from the (+)RNA·LS band of (*a*) and mixed with about 10 pmol of the R·*Hha*-digest of *HapC–Hae 1* in 0.6 M NaCl–0.06M Na citrate. The mixture was heated for 5 min at 100 °C and then held for 3 h at 55 °C. After cooling, the mixture was diluted, treated with 100 units of T1 RNase for 1 h at 37 °C, and electrophoresed for 3 h at 150 V on a 10% gel[12]. *d*, *HapC–Hae 1* (about 10 pmol) was mixed with about 30 pmol of RNA polymerase in a binding mixture (1 ml) containing 8 mM $MgCl_2$, 40 mM tris (pH 7.9), 0.15 M KCl, and 0.1 mM GTP, and the portion protected from DNase digestion was isolated after treatment with DNase I, as in ref. 12. The isolated fragment was heated for 5 min at 100 °C and added to a reaction mixture (1 ml) containing 8 mM $MgCl_2$, 50 mM KCl, 40 mM Tris (pH 7.9), 0.1 mM dithiothreitol, 20 μM each (α^{32}P)GTP and the other NTPs, 10 nmol AAAC and about 10 pmol RNA polymerase. After incubation for 3 h at 37 °C, the transcripts were isolated and mixed with 50 μg of fd phage DNA in 0.9 M NaCl–0.09 M Na citrate. The mixture was heated for 5 min at 100 °C and then held for 3 h at 51 °C. The fraction hybridised to fd phage DNA was isolated by using a Millipore filter and electrophoresed for 3 h at 150 V on a 15% gel containing 7 M urea, as in ref. 12.

The procedures for preparation of fd replicative form DNA (rfDNA) and *Escherichia coli* RNA polymerase holoenzyme, and the conditions used for digestion of DNA and gel electrophoresis have been described previously[8,9,12]. For preparation of *HapC–Hae 1*, *HapC* was first isolated from the R·*Hap* digest of fd rfDNA, redigested with R·*Hae*III, and then fractionated by gel electrophoresis. R·*Hap*, R·*Hae*III and R·*Hha* were respectively prepared from *Haemophilus aphirophilus*, *H. aegyptium* and *H. haemolyticus* by the procedures described previously[15]. For sequencing, RNA was synthesised with each of four (α^{32}P) nucleoside triphosphates (NTP), and hydrolysed with T1 RNase. The resulting nucleotides were fractionated by two-dimensional chromatography on a polyethyleneimine (PEI)–cellulose plate (Macherey–Nagel)[16]. Distribution of ^{32}P in spots was determined to estimate molar yields. To analyse the sequences of oligonucleotides, each spot was digested with either pancreatic RNase or U2 RNase and chromatographed using a two-dimensional system. The nucleotides produced were further digested with T2 RNase, and the distribution of ^{32}P in nucleotides determined. The starting nucleotides were not determined, since the nucleotides con-

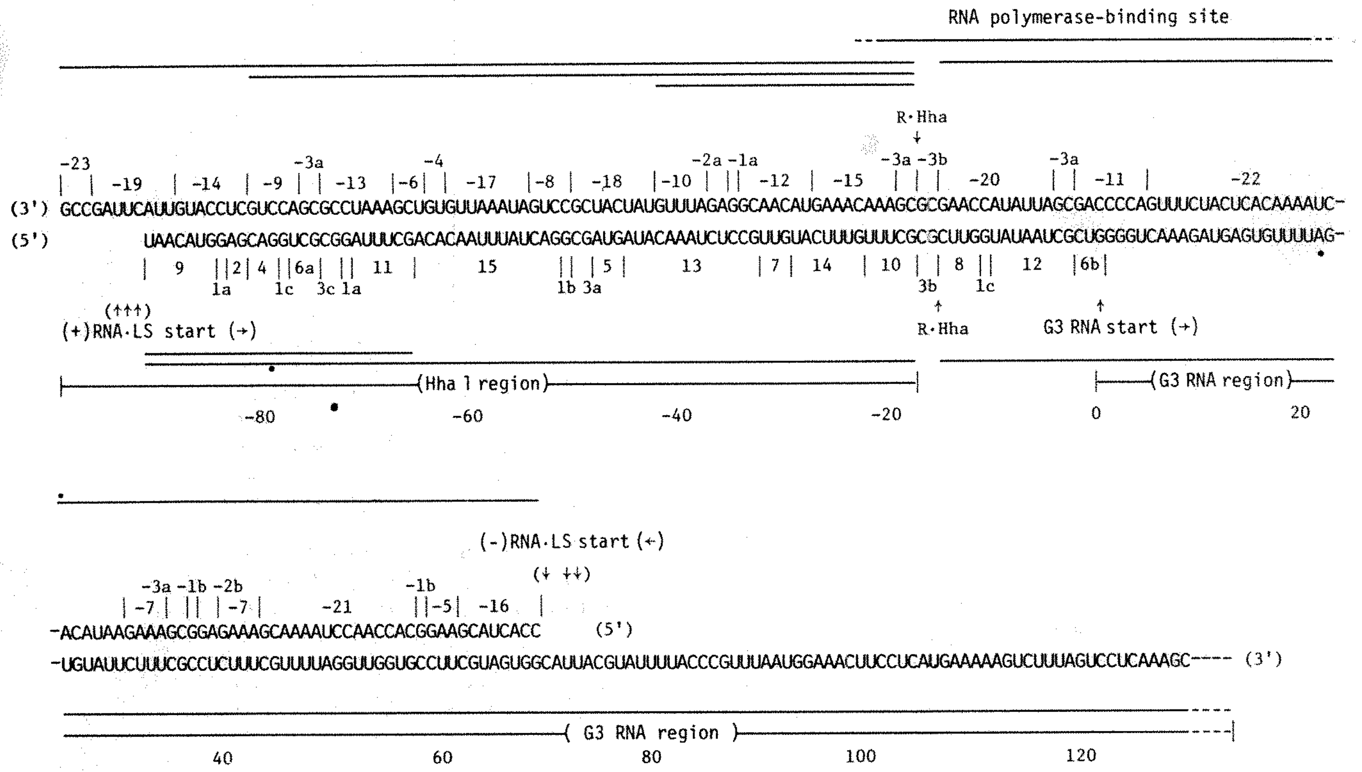
RNA polymerase-binding site
R·Hha
-23 -19 -14 -9 -3a -13 -6 -4 -17 -8 -18 -10 -2a -1a -12 -15 -3a -3b -20 -3a -11 -22
(3') GCCGAUUCAUUGUACCUCGUCCAGCGCCUAAAGCUGUGUUAAAUAGUCCGCUACUAUGUUUAGAGGCAACAUGAAACAAAGCGCGAACCAUAUUAGCGACCCCAGUUUCUACUCACAAAAUC-
(5') UAACAUGGAGCAGGUCGCGGAUUUCGACACAAUUUAUCAGGCGAUGAUACAAAUCUCCGUUGUACUUUGUUUCGCGCUUGGUAUAAUCGCUGGGGUCAAAGAUGAGUGUUUUAG-
9 1a 2 4 1c 6a 3c 1a 11 15 1b 3a 5 13 7 14 10 3b 8 1c 12 6b
(↑↑↑)
(+)RNA·LS start (→)
R·Hha
G3 RNA start (→)
(Hha 1 region)
(G3 RNA region)
-80 -60 -40 -20 0 20
(-)RNA·LS start (←)
(↓ ↓↓)
-3a -1b -2b -7 -7 -21 -1b -5 -16
-ACAUAAGAAAGCGGAGAAAGCAAAAUCCAACCACGGAAGCAUCACC (5')
-UGUAUUCUUUCGCCUCUUUCGUUUUAGGUUGGUGCCUUCGUAGUGGCAUUACGUAUUUUACCCGUUUAAUGGAAACUUCCUCAUGAAAAAGUCUUUAGUCCUCAAAGC---- (3')
(G3 RNA region)
40 60 80 100 120

Fig. 3 Arrangement of T1 RNase nucleotides produced from the low salt transcripts of *HapC–Hae 1*. Nucleotide numbers correspond to those given in Table 1. As the starting nucleotides were not identified, the possible start sites of (+)RNA·LS and (−)RNA·LS are indicated in parentheses. Sites corresponding to the R·*Hha* cleavage sites are indicated by arrows.

taining triphosphate ends stayed at the origin in the conditions used for chromatography.

Sequence in region preceding G3′ RNA start site

The nucleotides produced from G3′ RNA by digestion with pancreatic and T1 RNases have been analysed, and the sequence of 130 nucleotides from the 5′ end of G3′ RNA has been determined by analysis of its partial T1 RNase digest (Fig. 3 'G3 RNA region', our unpublished observation). The sequence contains the ribosome binding site number 1 of phage f1 messenger RNA[17] in the positions 96–120. Figure 4*a* and *b* shows fingerprints of T1 RNase nucleotides produced from G3′ RNA and (+)RNA·LS for comparison. By comparing the sequences of the products, (+)RNA·LS was found to contain 22 'extra' nucleotides. The positions of the extra nucleotides and their sequences are shown in Fig. 4*b* and Table 1*a*. These nucleotides were assigned to the region preceding the G3 RNA start site. To arrange these nucleotides, (+)RNA·LS was hybridised to the R·*Hha* digest of *HapC–Hae 1*. The regions protected by hybridisation to the DNA fragments, *Hha 1* and *Hha 2*, were isolated by gel electrophoresis after treatment with T1 RNase (Fig. 2*c*). The *Hha 1* and *Hha 2* regions were digested with T1 RNase and fractionated using a two-dimensional system. Among the 22 T1 RNase nucleotides of (+)RNA·LS assigned to the left of the G3 RNA region, 17 nucleotides arose from the *Hha 1* region, as indicated in Fig. 4*c*, and the remaining nucleotides from the *Hha 2* region. The order of the T1 RNase nucleotides in the *Hha 1* region was determined by analysis of its partial T1 RNase digests and of the T1 RNase nucleotides

Table 1 T1 RNase nucleotides produced from the low salt transcripts of *HapC–Hae 1*

a, Nucleotides from (+)RNA·LS

T1a: G A (2), T1b: G C (1), T1c: G U (2), T2: AG C (1), T3a: CG A (1), T3b: CG C (1), T3c: CG G (1), T4: CAG G (1), T5: AUG A (1), T6a: UCG C (1), T6b: CUG G (1), T7: UUG U (1), T8: CUUG G (1), T9: UAACAUG G (1), T10: UUUCG C (1), T11: AUUUCG A (1), T12: UAUAAUCG C (1), T13: (AU,AC)AAAUC(C,U)CG U (1), T14: UACUUUG U (1), T15: (AC,AC,AAUUU,AUC)AG G (1).

b, Nucleotides from (−)RNA·LS

−T1a: G A (1), −T1b: G C (2), −T2a: AG A (1), −T2b: AG G (1), −T3a: CG A (4), −T3b: CG C (1), −T4: UG U (1), −T5: AAG G (1), −T6: UCG A (1), −T7: AAAG A (2), −T8: CCUG A (1), −T9: ACCUG C (1), −T10: AUUUG U (1), −T11: ACCCCAG C (1), −T12: UACAACG G (1), −T13: AAAUCCG C (1), −T14: C(C,U)CAUG U (1), −T15: AAACAAAG U (1), −T16: CCACUACG A (1), −T17: AUAAAUUG U (1), −T18: UAUCAUCG C (1), −T19: UUACUUAG C (1), −T20: (AUU,AU,ACC)AAG C (1), −T21: C(ACC,AACC,U)AAAACG A (1), −T22*: (A_{10},C_6,U_6)UG A (1), −T23: CCG (>0.5)

*Pancreatic RNase digestion yields CA, CU, UA, UC, 2UU, ACA, 2ACU, AUC, AAUA, and AAAACA.

In *a*, nucleotides assigned to the region preceding the G3 RNA start site are listed. Nucleotide numbers correspond to those indicated in Fig. 4. Nucleotides produced from (−)RNA·LS are denoted by the minus sign. Nucleotides linked to the 5′ ends are underlined. Molar yields are indicated in parentheses.

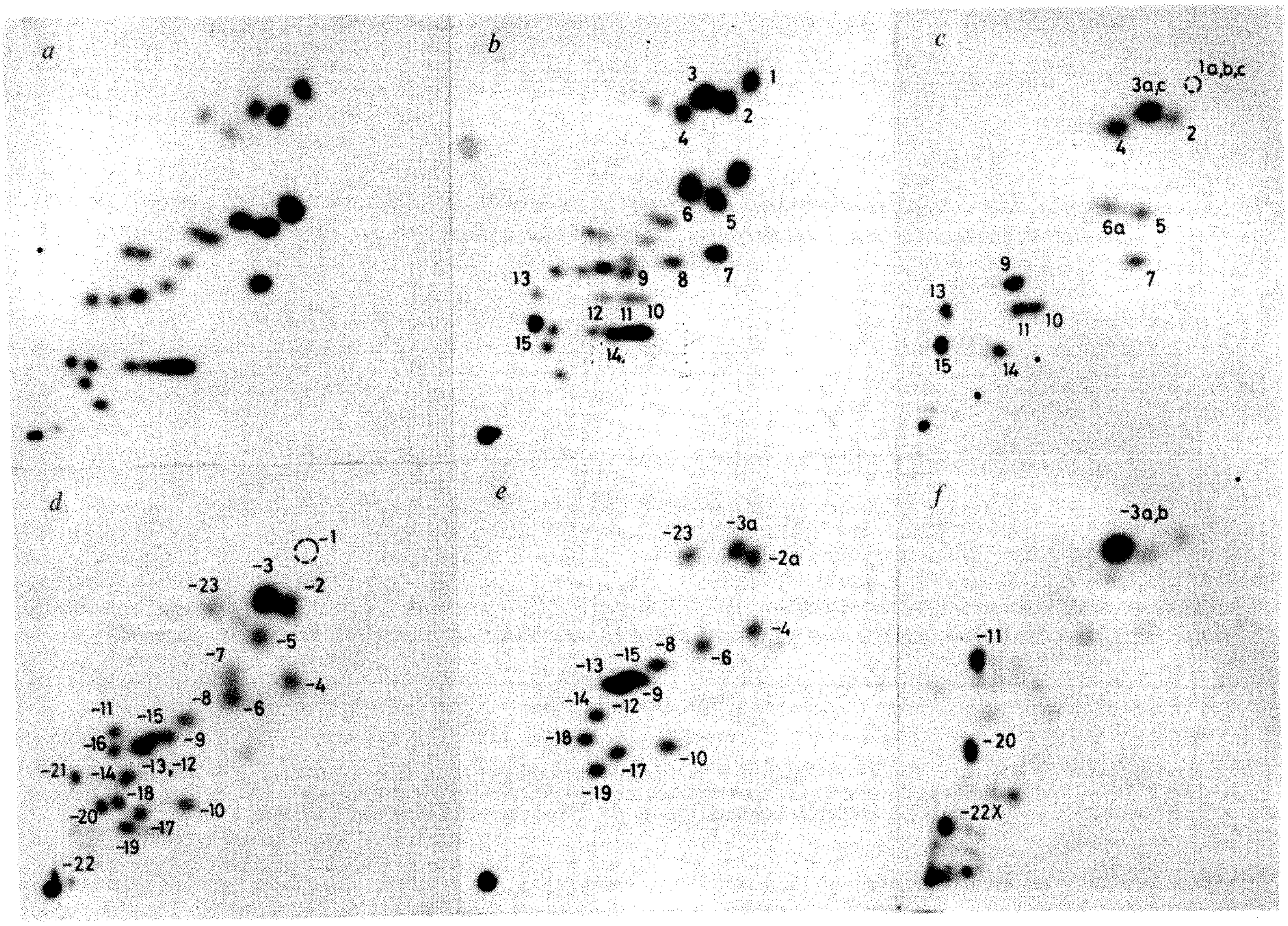

Fig. 4 Two-dimensional fingerprints of T1 RNase digests of *a*, G3′ RNA; *b*, (+)RNA·LS; *c*, portion of (+)RNA·LS hybridised to the DNA fragment *Hha 1* (Fig. 2*c*, *Hyb 1* region); *d*, (−)RNA·LS; *e*, portion of (−)RNA·LS hybridised to the DNA fragment *Hha 1*; and *f*, RNA formed on the RNA polymerase-protected fragment (Fig. 2*d*, B1 region). All RNAs have been synthesised with α-^{32}P-GTP, and the digests chromatographed on 20 × 20 cm PEI–cellulose plates, as in ref. 16. The sequences of the spots numbered are given in Table 1.

produced from the corresponding region of (−)RNA·LS (Fig. 4*e*). Although the sequences of T13 and T15 had not been deduced, a unique sequence was obtained for the *Hha 1* region by combining the sequence information on both strands (Fig. 3, '*Hha 1* region').

The five T1 RNase nucleotides remaining (T1c, T3b, T6b, T8 and T12) should be located between the *Hha 1* and G3 RNA regions. The sequence of the R·*Hha* cleavage site has been determined (ref. 18 and our unpublished observation). R·*Hha* cleaves DNA at the following sites:

↓
(5′)————pGpCpGpC————(3′)
(3′)————CpGpCpGp————(5′)
↑

This sequence should be located between the *Hha 1* and *Hha 2* regions. On the basis of this assumption and information on the nucleotides adjacent to G, the order of the five T1 RNase nucleotides can be deduced to be either (*a*) T3b:T8:T1c:T12:T6b or (*b*) T3b:T6b:T1c:T12:T8.

The T1 RNase nucleotides produced from (−)RNA·LS are shown in Fig. 4*d* and Table 1*b*, in which nucleotides from (−)RNA·LS are denoted by the minus sign. As indicated in Fig. 1, the sequence of (−)RNA·LS should be complementary to the left part of (+)RNA·LS. According to this assumption we arranged the T1 RNase nucleotides of (−)RNA·LS on the sequence of (+)RNA·LS, and 28 nucleotides were successfully ordered on the *Hha 1* and G3 regions. Although the sequences of −T14, −T21 and −T22 had not been deduced, analysis of their pancreatic RNase digests indicates that these fragments can only fit in the indicated regions (Fig. 3). −T23 was assigned to the left end of the sequence, as the yield was less than 1 mol (Table 1*b*). Only three T1 RNase nucleotides remain (−T3a, −T3b and −T20). These nucleotides were assigned to the region between the *Hha 1* and G3 RNA regions. As described above, the five T1 RNase nucleotides of (+)RNA·LS which had been assigned to the corresponding region can be ordered into two alternative sequences ((*a*) or (*b*)). The three T1 RNase nucleotides from (−)RNA·LS are only arranged on the sequence of (*a*), (see Fig. 3). This arrangement was further confirmed by analysis of the portions of (−)RNA·LS hybridised to the *Hha* fragments and of their partial T1 RNase digests. In Fig. 4*e*, the T1 RNase nucleotides produced from the *Hha 1* region of (−)RNA·LS are shown: all the nucleotides assigned to the left of the R·*Hha* cleavage site are seen. The regions covered by other RNA fragments analysed are indicated by the sidelines on the sequence in Fig. 3.

Sequence of RNA polymerase binding site

The initiation complex was formed with *HapC–Hae 1* in the presence of 0.15 M KCl, and the polymerase-protected fragment was isolated after DNase digestion. The fragment was denatured and RNA synthesis was carried out using conditions described previously[12]. AAAC was added as a primer, because RNA synthesis was significantly stimulated by this primer. The synthesised products were fractionated by hybridisation to fd phage DNA, followed by gel electrophoresis. The T1 RNase nucleotides produced from the largest RNA band (Fig. 2*d*, B1 region) is shown in Fig. 4*f*. Only six major nucleotides were identified, of which five were −T3a (2 mol), −T3b, −T11 and

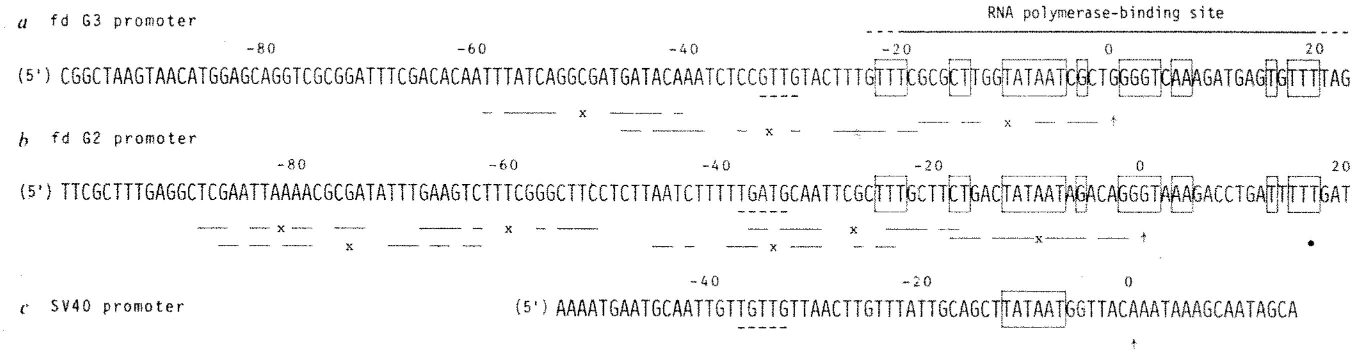
a fd G3 promoter
RNA polymerase-binding site
-80 -60 -40 -20 0 20
(5') CGGCTAAGTAACATGGAGCAGGTCGCGGATTTCGACACAATTTATCAGGCGATGATACAAATCTCCGTTGTACTTTGTTTCGCGCTTGGTATAATCGCTGGGGTCAAAGATGAGTGTTTTAG
x
b fd G2 promoter
-80 -60 -40 -20 0 20
(5') TTCGCTTTGAGGCTCGAATTAAAACGCGATATTTGAAGTCTTTCGGGCTTCCTCTTAATCTTTTTGATGCAATTCGCTTTGCTTCTGACTATAATAGACAGGGTAAAGACCTGATTTTTGAT
x
c SV40 promoter
-40 -20 0
(5') AAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCA

Fig. 5 Comparison of sequences of *a*, fd G3 promoter; *b*, fd G2 promoter, and *c*, a promoter for *E. coli* RNA polymerase on SV40 DNA. The sequences of *b* and *c* were taken from refs 7 and 2. In *a* and *b*, nucleotides common to the polymerase binding sites of both fd promoters are boxed, and the regions with twofold symmetry are indicated by underlining (*x*, axis of symmetry). In *c*, the homologous sequence in the polymerase binding site is boxed. Arrows indicate the starting points of transcription.

−T20 in the T1 RNase products of (−)RNA·LS. The other (−T22X) contained the primer at 5′ end: the result of nearest neighbour analysis indicated that this nucleotide just fits in the region between the sixth and twentieth nucleotides of G3 RNA. It is therefore reasonable to conclude that the RNA analysed had been initiated with the primer in the middle of the −T22 region and terminated within the −T15 region on the sequence of (−)RNA·LS, as indicated in Fig. 3. The size of RNA is about equal to the approximate size of the DNA fragment isolated as the polymerase binding site. The sequence of the polymerase binding site, inferred from the RNA sequence, contains the initiation point for transcription near the centre, in accordance with the observations on other RNA polymerase binding sites[12–14].

Sequence features of fd promoter

The sequence in the promoter region for G3 RNA (G3 promoter) inferred from analysis of the low salt transcripts, is shown in Fig. 5*a*. All the information needed for promoter function should be contained in this sequence. The sequence was first compared with the sequence of fd G2 promoter determined previously (Fig. 5*b*)[7]. The longest sequence common to the two similar fd promoters, however, was only TATAAT. It is also seen that when both the promoter sequences are aligned with reference to the TATAAT sequence, a relatively high degree of homology is obtained in the polymerase binding site, as indicated in boxes in Fig. 5*a* and *b*. Any other alignment does not yield such a homology in any region. We previously noted that the G2 promoter contains regions with twofold rotational symmetry, with one located in the non transcribed region of the polymerase binding site (Fig. 5*b*)[7,12]. The G3 promoter also contains some symmetrical regions (Fig. 5*a*). Comparison of the symmetrical regions, however, does not reveal a common pattern.

In a previous paper[7], we noted that, among the promoters already sequenced[1–6], the G2 promoter sequence bears some resemblance to the sequence of a promoter for *E. coli* RNA polymerase on SV40 DNA (Fig. 5*c*) (TTNTTGNTG at around the −35th position and TATAAT in the polymerase binding site). The G3 promoter also contains some regions homologous to the SV40 promoter (GTTGT and TTGTT at positions −20 and −40 and TATAAT in the polymerase binding site). The sequence common to all three promoters, however, is only the TATAAT sequence. As has been noted by Pribnow[14], the other promoters also contain the sequences homologous to TATAAT in the corresponding region. The result therefore strongly suggests that the TATAAT sequence has an important role for the formation of the initiation complex. It is unlikely, however, that RNA polymerase directly binds to the RNA initiation site by recognising this TATAAT sequence, because the R·*Hha* cleavage of the G3 promoter five base pairs upstream from the TATAAT sequence destroys the ability to form the initiation complex. It has also been shown that RNA polymerase does not initiate RNA synthesis correctly on the isolated polymerase-binding fragment[14,19]. The exact size of the promoter is not known. In the *lac* and λ promoters, some promoter mutations locate approximately 35 base pairs upstream from the RNA start site[4,20,21]. In the λ promoters, the R·*Hin*dII cleavage site which destroys the promoter function is located 33 base pairs from the RNA start site[1,5,6]. These observations all suggest that some regions preceding the polymerase binding site are essential for promoter function. It is most likely that RNA polymerase first recognises some specific structure in such a region (recognition site) and then forms an initiation complex at the region containing the TATAAT sequence[4,14,21].

The question remains as to the basic structure at the recognition site. One possibility is that some specific sequence is involved in the primary recognition of promoter by RNA polymerase. It has been noted that both the λ and SV40 promoters contain TGTTG about 35 base pairs upstream from the RNA start site[21]. As indicated by broken lines in Fig. 5*a* and *b*, the G2 promoter contains TGATG and the G3 promoter GTTG in the corresponding region, suggesting that this sequence may have some relation to the structure of the recognition site. The *lac*[4] and tyrosine transfer RNA[24] promoters, however, do not contain this sequence. The other possibility for consideration is derived from the present finding that, except for the polymerase binding site, there was little sequence homology between two similar fd promoters. It is known that RNA polymerase has strong affinity towards single-stranded regions of DNA[22,23]. At low salt concentrations, RNA polymerase binds many sites of DNA[22] and initiates RNA synthesis at additional sites, as indicated in this and previous papers[7]. These lines of evidence would lead to a view that RNA polymerase primary interacts with regions of low transition temperature, instead of recognising a specific sequence; and if the TATAAT sequence locates at the neighbouring site, the polymerase forms a stable initiation complex. Such a primary interaction site could be either the region of high AT content or the region with twofold symmetry, as the helical structure of these regions is assumed to be unstable. In support of this possibility, all the promoters sequenced seem to have some AT-rich regions and the fd promoters contain the regions with twofold symmetry in the region preceding the polymerase-binding site, although the location and size of such regions are different with promoters. With either possibility discussed above, more information is still needed to define the basic structure at the recognition site.

Finally, we compared the sequences of two fd promoters with similar properties and found little sequence homology, except for the region in which RNA polymerase forms an initiation complex. As other lines of evidence indicate that the promoter recognition by RNA polymerase takes place at a region preceding the initiation complex site, our finding implies that a unique sequence is not involved as a key element in the promoter recognition.

Received October 24, 1975; accepted February 9, 1976.

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Intracellular forms of Epstein–Barr virus DNA in human tumour cells *in vivo*

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Tumour biopsies from Burkitt lymphoma patients, as well as human nasopharyngeal carcinoma cells growing in athymic nude mice, contain Epstein–Barr virus DNA as covalently closed circular DNA. In addition, integrated viral DNA sequences seem to be present.

EPSTEIN–BARR virus (EBV) is of great interest at present as the first virus of possible aetiological significance for certain malignant tumours in man. Two unusual human tumours are uniquely associated with EBV, the African form of Burkitt lymphoma and low-differentiated nasopharyngeal carcinoma. Patients with these tumours always have elevated serum titres against EBV-associated antigens[1,2]. Moreover, the actual tumour cells are those transformed by the virus, as they contain several copies of EBV DNA and express an EBV-associated nuclear antigen (EBNA) but lack detectable virus particles[2–5]. Although formal proof that the virus is oncogenic in man will be difficult to obtain, the notion that EBV is a tumour virus is supported by the recent findings that injection of the virus into cotton-top marmosets or owl monkeys induces fatal lymphomas[6,7], and that the virus transforms normal human B lymphocytes into immortal lymphoblastoid cell lines with great efficiency[8]. Further, in humans with certain rare forms of inherited immunodeficiency, infectious mononucleosis caused by EBV may proceed into a malignant lymphoma instead of having its usual appearance as a self-limiting lymphoproliferative disease[9,10].

Insight into the relationship between EBV and its host may be gained from studies on the state of the virus genome within the transformed cell. Such investigations have previously been limited to human lymphoid cell lines, in particular Raji cells. This line was derived from a Burkitt lymphoma biopsy over 10 yr ago[11]. A Raji cell does not produce EBV particles, but it contains 50–60 genome equivalents of EBV DNA[12]. Most of this intracellular EBV DNA is present in free form and can be separated from the larger cellular DNA by glycerol gradient centrifugation in both alkaline and neutral solution[13,14]. This non-integrated EBV DNA has been purified and shown to have a circular conformation[15,16], with both covalently closed and nicked circular DNA molecules present after isolation. Thus, the non-integrated intracellular virus DNA differs structurally from the linear duplex DNA present in virus particles, although both these DNA forms are of the same molecular weight within experimental error. In addition, integrated sequences of EBV DNA have been found in Raji cells, but it is not known if they represent complete virus genomes[17]. The isolated DNA "molecules" comprised of both viral and cellular DNA usually contain single-strand interruptions, but the actual joints between the viral and cellular DNA are alkali stable[18].

It has not been known if both integrated and circular forms of EBV DNA are also present in tumour cells *in vivo* or if one of these forms was a secondary consequence of *in vitro* cultivation. The studies on the intracellular states of EBV DNA are extended here to tumour biopsies.

Circular EBV DNA in nasopharyngeal carcinoma cells

Biopsies from patients with low differentiated nasopharyngeal carcinoma contain EBV DNA, which is located in the nuclei of the epithelial tumour cells[19,20]. Such biopsies are usually small and heavily infiltrated with lymphocytes that do not contain EBV DNA, and they are therefore less

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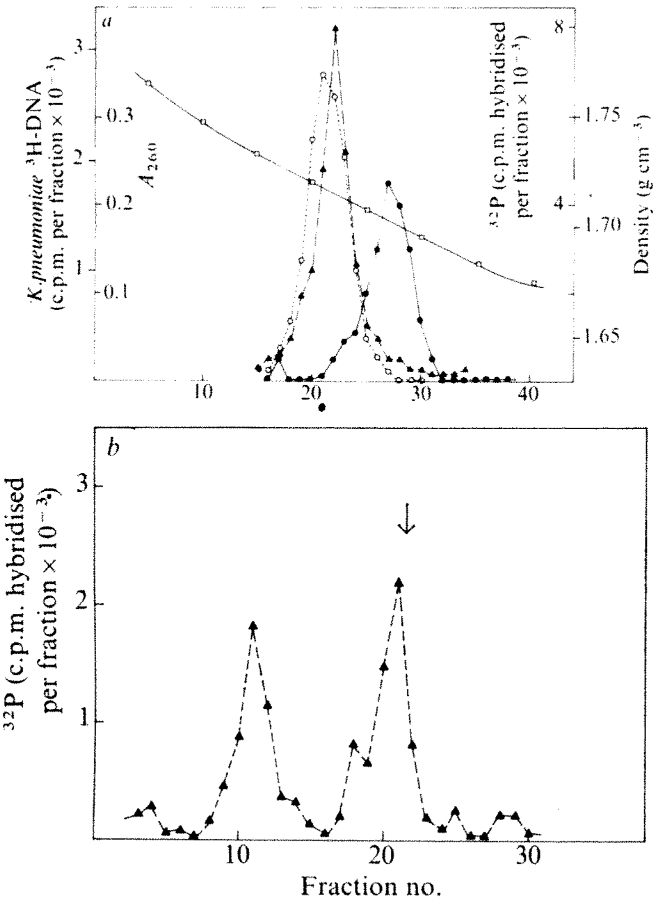
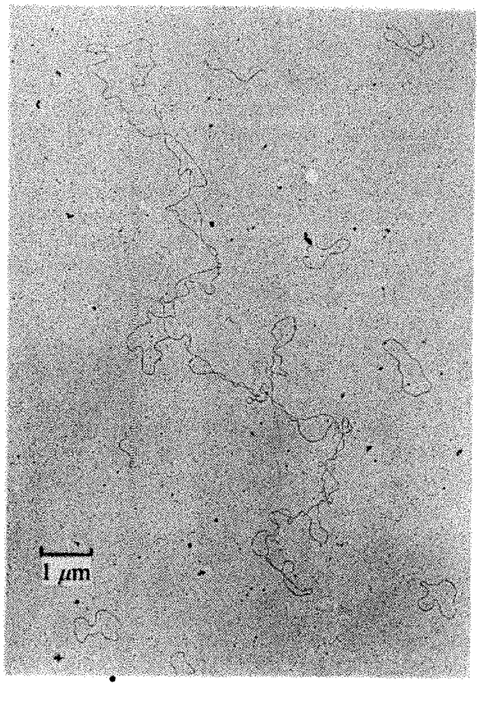


Fig. 1 Separation of EBV DNA and cellular DNA in lysates of nasopharyngeal carcinoma cells. A human tumour (M.M., 1.5 g) growing in a nude mouse was excised, finely minced with a pair of scissors in 6 ml of 0.15 M NaCl–0.01 M Tris-HCl–0.001 M $MgCl_2$–0.0005 M $CaCl_2$ (*p*H 7.2) at 0 °C, and the cells were disintegrated by twenty strokes in a Teflon-glass hand homogeniser. Six ml of 3% Sarkosyl–0.075 M Tris-HCl–0.025 M EDTA (*p*H 9.0) were then added, and the viscous lysate was brought to 20 °C. After 40 min, 3 ml of 0.5% Pronase (preincubated for 2 h at 37 °C in 0.05 M Tris-HCl (*p*H 7.5) before use) were added, and the mixture was incubated for 2 h at 37 °C. It was then diluted with 100 ml 0.05 M Tris-HCl (*p*H 8.5), and supplemented with a density marker (*Klebsiella pneumoniae* ^{3}H-DNA, $\rho = 1.717$ g cm^{-3}) and solid CsCl to a final density of 1.714 g cm^{-3}. The material was centrifuged in eight 19-ml aliquots overlayered with paraffin oil in a Spinco 60 Ti rotor at 33,000 r.p.m. for 65 h at 20 °C. The gradients were collected through a large hole in the bottom of the tube with a closed system collection device[16] in 0.4-ml fractions. A gradient profile is shown in *a*. In each fraction, the cellular DNA was determined by A_{260} measurements, the DNA density marker by ^{3}H radioactivity, and EBV DNA sequences by hybridisation of filter-bound DNA with ^{32}P-labelled EBV complementary RNA as described[16]. The density of every fifth fraction was determined by refractometry. ●, A_{260}; ○, *K. pneumoniae* ^{3}H-DNA; ▲, EBV DNA; □, density. High density DNA from a second nasopharyngeal carcinoma (J.G.) was isolated from two gradients of the type shown in frame *a* (fractions 20–23), dialysed against 0.1 M NaCl–0.01 M Tris-HCl (*p*H 8.0)–0.001 M EDTA, concentrated twofold by packing the dialysis bag in dry polyethylene glycol 6000 for 50 min, mixed with a size marker, phage T4 ^{14}C-DNA (0.6 μg, 9,000 c.p.m.), and applied to a 37-ml glycerol gradient (10–30%) containing 1 M NaCl–0.02 M Tris-HCl (*p*H 8.0)–0.001 M EDTA. The DNA was centrifuged in a Spinco SW27 rotor at 25,000 r.p.m. for 220 min at 21 °C, and the centrifuge profile is shown in *b*. 1-ml fractions were collected, the T4 DNA marker (arrow) was localised by radioactivity measurements, and the EBV DNA sequences (▲) by hybridisation with EBV ^{32}P-cRNA. The direction of sedimentation is from right to left.

suitable for biochemical work. The tumour cells carrying EBV, however, continue to grow after implantation to congenitally athymic (nude) mice, whereas the infiltrating lymphoid cells disappear[20]. We have used such human tumours growing in nude mice to investigate the intracellular state of EBV DNA in nasopharyngeal carcinoma cells.

The M. M. and J. G. carcinomas contain about 80 and 150 EBV genome equivalents per tumour cell, respectively[20]. Tumour-carrying nude mice were transported from the Houston laboratory to Stockholm. The animals were killed, and the tumours were immediately excised and used for the preparation of high molecular weight DNA. The DNA was then fractionated by CsCl density gradient centrifugation and collected avoiding shearing forces. The EBV DNA sequences were localised by hybridisation of the individual gradient fractions with ^{32}P-labelled EBV complementary RNA. The experimental techniques are the same as those previously used with Raji cells[15–18]. A typical gradient profile is shown in Fig. 1. EBV DNA has a higher guanine–cytosine content than cellular DNA, and most of the virus DNA sequences are separated from the bulk of the cellular DNA and form a peak at a density of 1.716 g cm^{-3}. The EBV DNA peaks from two CsCl gradients were pooled and further fractionated by neutral glycerol gradient centrifugation together with phage T4 ^{14}C-DNA as an internal size marker[21]. Linear EBV DNA from virus particles sediments at 58–59S in these conditions[15,16]. In contrast, the intracellular EBV DNA sequences from the J. G. and M. M. tumours were found as two distinct peaks at about 101S and 64S (Fig. 1). The two forms of intracellular EBV DNA from the nasopharyngeal carcinoma cells sediment at 1.7 and 1.1 times the rate of linear EBV DNA from virus particles, indicating that they correspond to covalently closed circular virus DNA molecules and open circular virus DNA molecules, respectively[22]. The presence of large covalently closed circular DNA molecules was directly confirmed by electron microscopy

Fig. 2 Electron micrograph of a large, covalently closed circular DNA molecule from a nasopharyngeal carcinoma tumour (M.M.). The EBV DNA was partly purified by CsCl density gradient centrifugation and glycerol gradient centrifugation. The DNA sedimenting at 95–110S in the latter type of gradient was pooled and dialysed against 0.05 M NaCl–0.01 M Tris-HCl (*p*H 8.1)–0.005 M EDTA and spread by a modification of the microdiffusion technique avoiding the use of formaldehyde[16,25]. The small DNA circles in the figure are phage PM2 DNA molecules. In order to partially untwist the supercoiled molecules, 4 μg ml^{-1} ethidium bromide was included in the DNA–cytochrome *c* solution. For length measurements, however, only preparations without ethidium bromide were used.



(Fig. 2). Such large DNA circles have previously been found in corresponding fractions from Raji cells, but are not detected in EBV-negative human cell lines[16]. The covalently closed circular DNA molecules from nasopharyngeal carcinoma cells (J. G.) were converted to an open circular form by X-ray treatment, mixed with open circular DNA from phage PM2 (a gift from Dr R. Streeck) as an internal size marker, and used for contour length measurements on electron micrographs[16]. These EBV DNA circles were found to be 16.2–16.3 times larger than PM2 DNA molecules, corresponding to an approximate molecular weight of 104×10^6. In previous measurements on circular EBV DNA from the Burkitt lymphoma derived cell line Raji, the molecules were found to be 16.6 times larger than PM2 DNA[16], and the covalently closed and open circular forms of the virus DNA has sedimentation coefficients of 103–105S and 65S, respectively. Thus, both the contour length measurements and the sedimentation velocity data indicate that the circular EBV DNA in nasopharyngeal carcinoma cells may be slightly smaller than that in Raji cells, suggesting the presence of a different EBV subgroup or a small deletion of the virus DNA in the epithelial cells.

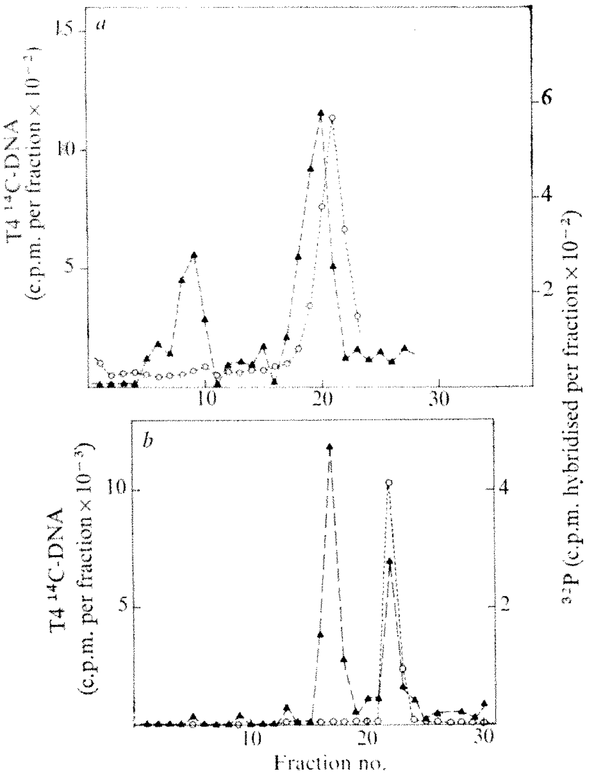


Fig. 3 Fractionation of high density DNA from a Burkitt lymphoma biopsy. The A.O. biopsy (5 g) was minced with scissors, and the material was suspended in ten volumes tissue culture medium by shaking. After 10 min at 21 °C, the material that had settled to the bottom, which consisted largely of stroma, was discarded, and suspended cells were collected by low speed centrifugation and washed in phosphate-buffered saline. The cells (10^7 cells ml^{-1}) were lysed by addition of Sarkosyl–EDTA and Pronase, and the DNA was fractionated by CsCl gradient centrifugation as in Fig. 1*a*. The high density DNA was collected and cosedimented with phage T4 DNA on a glycerol gradient as in Fig. 1*b*. The results from the latter gradient are shown in frame *a*. ▲, EBV DNA, as determined by hybridisation with EBV cRNA; ○, T4 DNA. The fractions 25–31 contained the density marker DNA from the CsCl purification step. The 105S DNA from one neutral glycerol gradient was recovered, mixed with linear ^{14}C-DNA (from phage T4), and dialysed against 1 M NaCl–0.02 M Tris-HCl (*p*H 8.0)–0.001 M EDTA for 20 h, followed by 0.05 M NaCl–0.02 M Tris-HCl (*p*H 8.0)–0.001 M EDTA for 3 h. The material was concentrated two- to threefold by packing the dialysis bag in dry polyethylene glycol 6000 for 1 h, followed by an additional 3-h dialysis against the last buffer. The DNA sample (2.2 ml) was then supplemented with 0.2 ml 0.6% propidium diiodide in 0.01 M Tris-HCl (*p*H 8.0) and 2.25 g solid CsCl. Centrifugation was carried out in a 5-ml tube overlayered with paraffin oil in a Spinco SW 50.1 rotor at 40,000 r.p.m. for 48 h at 21 °C. Fractions (0.1 ml) were collected from the bottom of the tube, and the fractions were freed from the dye by four extractions with two volumes of 90% isopropanol. Data are shown in frame *b*. ○, T4 DNA; ▲, EBV DNA, as determined by hybridisation with EBV cRNA.

Circular EBV DNA in Burkitt lymphoma biopsies

Burkitt lymphoma patients were treated by tumour excision in Nairobi. Tumour material was transported on ice to Stockholm, and was available for experimentation about 24 h after excision. Burkitt lymphoma biopsies are more homogeneous than nasopharyngeal carcinoma biopsies, and occasionally large specimens with a high proportion of living tumour cells (>80%) became available. Two such tumours were investigated, an ovary tumour from a 9-yr-old girl (A.O.) and a tumour excised from the submandibular region of a 4-yr-old boy (W.J.).

High molecular weight DNA was extracted from the Burkitt lymphoma cells and fractionated on CsCl gradients and neutral glycerol gradients. The results were quite similar to those obtained with the nasopharyngeal carcinoma cells. With material from one of the biopsies (A.O.), two distinct peaks of EBV DNA at 105S and 65S were observed by hybridisation of the glycerol gradient fractions with EBV complementary RNA (cRNA) (Fig. 3), representing covalently closed circular EBV DNA and open circular EBV DNA. In the other biopsy, a single peak of 65S EBV DNA was found, corresponding to open circular DNA. The apparent absence of covalently closed circular EBV DNA in the latter case may have been due to the introduction of single-strand breaks into the viral DNA after tumour excision, either during the transport to Stockholm or during the preparation of the lysate from the tumour.

The 105S and 65S EBV DNA fractions from the A.O. biopsy were further characterised by equilibrium density gradient centrifugation in propidium diiodide–CsCl gradients. Covalently closed circular duplex DNA has a higher density in such gradients than open circular or linear DNA[23]. Most of the EBV DNA (70%) from the 105S region banded as a distinct peak at a position typical of covalently closed circular DNA (Fig. 3), as estimated from parallel control experiments with polyoma virus DNA. The rest of the EBV DNA (30%) was found at the position of open circular and linear DNA, and was presumably due to DNA damaged during the preparation procedures. All of the 65S EBV DNA banded as open circular or linear EBV DNA.

Integrated EBV DNA

Part of the EBV DNA sequences present in Raji cells band at an anomalously light density in CsCl gradients. The densities of these viral DNA sequences remain unchanged after treatment with Pronase, sodium dodecyl sulphate, and phenol, but they appear at a heavier density after reduction of the size of the DNA preparations by shearing forces. These data indicate that integrated EBV DNA sequences are present in Raji cells[17,18]. A similar experimental approach has now been used with the tumour biopsy material studied here. In the nasopharyngeal carcinoma DNA shown in Fig. 1*a*, a small part of the total EBV DNA sequences is present under the peak of cellular DNA. To remove EBV DNA tailing into the low density region, this DNA (fractions 26–29 in Fig. 1*a*) was rebanded on a second CsCl gradient. The DNA banding at a density of 1.698–

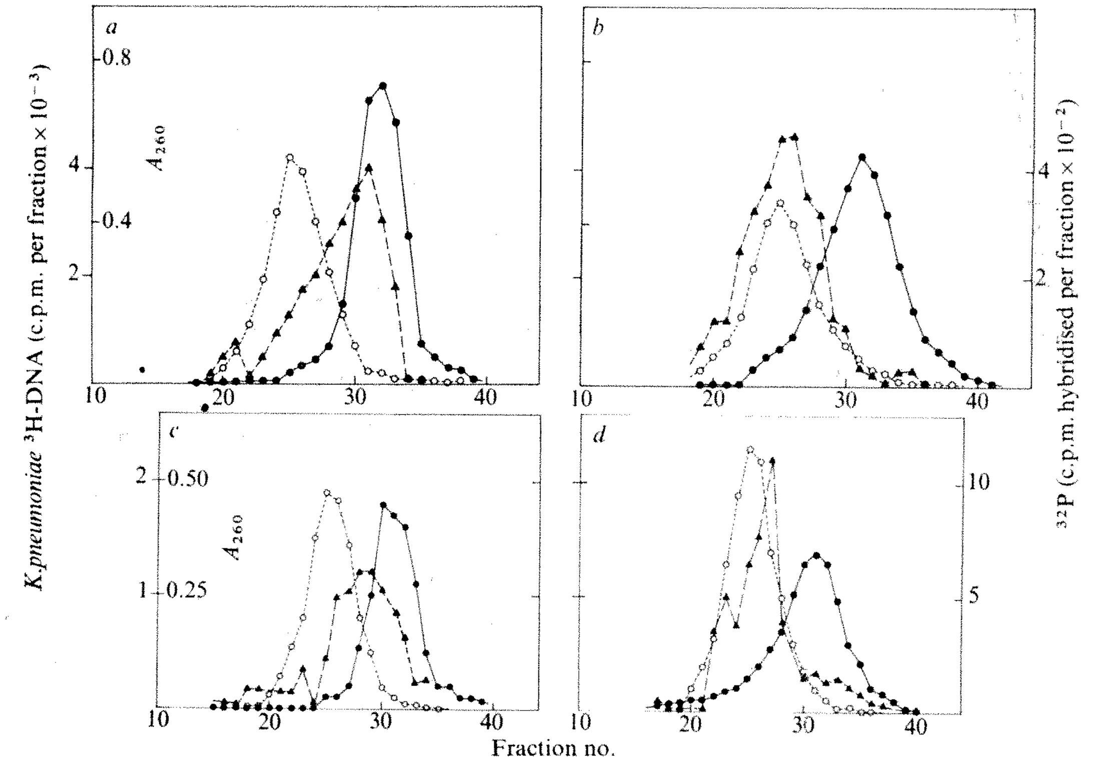


Fig. 4 Third sequential fractionation by CsCl gradient centrifugation of cellular density DNA from tumour cells. For studies on nasopharyngeal carcinoma cells, fractions 26–29 in Fig. 1*a* were rebanded in CsCl in the same experimental conditions, and the main peak of A_{260} absorbing material from the second gradient was pooled, mixed with a density marker (*K. pneumoniae* ³H-DNA), and divided in two parts. One part was sheared by three rapid passages through a No. 25 hypodermic syringe needle. The two DNA solutions were individually rebanded in CsCl in the same experimental conditions. *a*, Unsheared DNA; *b*, Sheared DNA. ●, A_{260}; ○, *K. pneumoniae* ³H-DNA; ▲, EBV DNA sequences determined by hybridisation with EBV ³²P-cRNA. The same experimental design was used with Burkitt lymphoma biopsy cells, and the data obtained with the A.O. biopsy are shown in frames *c* and *d*. After purification of cellular density DNA by two consecutive CsCl gradient centrifugation steps, the material was divided in two parts, and the size of the DNA of one part was reduced twentyfold by shearing before rebanding and hybridisation. *c*, Unsheared DNA, and *d*, sheared DNA. Symbols as in *a* and *b*.

1.708 g cm^{-3} in this gradient was supplemented with a density marker (*Klebsiella pneumoniae* ³H-DNA) and divided in two equal parts. One part was again centrifuged to equilibrium in neutral CsCi. Most of the remaining EBV DNA sequences in this DNA fraction now banded at a peak density of about 1.704 g cm^{-3}, but some EBV DNA sequences of heavier densities were still present (Fig. 4*a*). The other part of the material was reduced in size by shear treatment, and then banded in CsCl in the same conditions. In this case, the EBV DNA sequences were released to heavier densities. (Fig. 4*b*). This is the expected result if large DNA molecules containing both EBV DNA sequences and cellular DNA sequences had been fragmented by the shear treatment.

The DNA preparations from the two Burkitt lymphoma biopsies were also studied by the same techniques, and the results were essentially identical to those obtained with the nasopharyngeal carcinoma material. With both biopsies, a low density fraction of EBV DNA sequences could be isolated by three consecutive bandings in CsCl, and these viral DNA sequences were shifted to heavier densities by reduction of the size of the DNA. The data obtained with one of the biopsies (A.O.) are shown in Fig. 4. It is clear that this type of low density EBV DNA, which was first detected in lymphoid cell lines and presumably consists of integrated sequences of virus DNA, is present also in tumour biopsies of Burkitt lymphoma and nasopharyngeal carcinoma origin. Thus, the integrated viral sequences can not be ascribed to chromosome translocation events or other artefacts of long term *in vitro* culture.

Episomal state of latent EBV

In bacteria, sex factors and certain prophages can be carried both in an integrated state and as stable extrachromosomal elements in the form of circular DNA molecules. The discovery of circular EBV DNA molecules in virus-transformed human cells, free from virus particles, strongly indicates that episomes also occur among mammalian cells. Little information is available at present on the carrier states of other large animal viruses, and it seems possible that all herpesviruses may be carried as episomes during latency. The relationship between the non-integrated EBV DNA circles and the integrated EBV DNA sequences found in transformed cells remains a subject for future study.

In addition to the investigations on tumour cells, we have recently shown (to be published) that circular EBV DNA molecules are also found in EBV DNA-carrying lymphoblastoid cell lines derived from normal individuals and from infectious mononucleosis patients. A lymphoblastoid cell line derived from a normal individual also contained EBV DNA sequences with the properties of integrated DNA. No clear difference has so far been observed between the carrier state of EBV in cell lines from normal individuals and in tumour cells. A simple model in which either integrated EBV DNA sequences or circular EBV DNA molecules would only be found in tumour cells is not in agreement with the available data. It should be noted, however, that EBV-carrying lymphoblastoid cell lines derived from human blood of healthy donors have malignant potential, as they cause tumours on implantation in nude mice[24].

This investigation was supported by the US National Cancer Institute, the Swedish Cancer Society, the Swedish Natural Science Research Council, and the Stehlin Foundation for Cancer Research. The electron microscope was purchased with grants from the Swedish Work Environment Fund and the Trustees of the Knut and Alice

Wallenberg Foundation. C. K.-D. was the recipient of a fellowship from the Deutsche Forschungsgemeinschaft. G.W.B. had a short term fellowship from EMBO and is supported by the Deutsche Forschungsgemeinschaft.

Received September 29, 1975; accepted February 9, 1976.

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letters to nature

New H_2O celestial sources associated with H II regions in the Southern Hemisphere

We report here the first results of a nearly complete, high-sensitivity, water vapour survey of galactic H II regions situated in the Southern Hemisphere.

A typical upper limit of about 40 Jy (1 Jy $= 10^{-26}$ W m^{-2} Hz^{-1}) was attained at the $6_{16} \rightarrow 5_{23}$ rotational transition line of the water vapour molecule (that is, a frequency of 22,235.08 MHz).

A new ruby travelling wave K-band maser receiver[1] was used at the 13.7-m Itapetinga radio telescope[2], Atibaia, São Paulo, Brazil, between November 4 and 8, 1975. The Dicke-switched system temperature was typically 350 K, including radome contribution (less than 10 K)[3] and sky contribution (less than 100 K during this season)[3]. The back-end section of the receiver consisted of a 43-channel spectrograph with 100-kHz filters. Observations were made using a combined beamswitching and 'on–on' technique.

The 13.7-m Itapetinga radio telescope has an absolute pointing accuracy better than 15″ arc, which is negligible in comparison with its 4.5′ arc beamwidth at that frequency. The aperture efficiency was redetermined using Virgo A (assuming 21.4 Jy)[4] and Venus (brightness temperature of 420 K)[5] as calibrators, and was found to be 34.5 Jy K^{-1} (that is, 54% efficiency).

Observations were carried out during the worst season for millimetric work at Itapetinga. Frequent measurements of atmospheric absorption were necessary. Optical depths were determined using the sky emission method[3], and ranged from 2.61 to 4.34 dB. All measurements were corrected for atmospheric and radome absorption, the latter being 23% at 33.2 GHz.

The most complete published surveys of new H_2O celestial sources in the Southern Hemisphere are those by Johnston *et al.*[6], and by Caswell *et al.*[7]. They reported sources having fluxes larger than or equal to about 200 Jy.

We examined all 47 of the individual H II regions with 5 GHz continuum peak brightness levels of 5 K or more, as reported by Goss and Shaver[8], and all 24 regions for which OH main-line emission has been found[9-16].

Observations were made at reported positions (Table 1), and no attempt was made to find improved H_2O positions.

Thirteen new H_2O celestial masers were found (Table 1). The spectrograph was centred at the velocities of associated OH lines, when known, otherwise at H109α recombination lines[12]. The search width for each observation was in general ± 29 km s^{-1}.

Of the sources associated with OH emission, all except H_2O 0.55−0.85 had previously been examined[6,7] for H_2O emission. It is generally accepted that sources having OH main-line emission are also likely to show H_2O emission[13]. In Fig. 1*a* we show the spectrum for the strongest source discovered in this class. Another interesting H_2O source, with three line components, is H_2O 0.55−0.85. This has been identified with RCW142 (Fig. 1*b*), a dense molecular cloud near the galactic centre[11].

The seven new H_2O sources associated with H II regions that do not exhibit OH emission, suggest the existence of a new class of celestial water vapour masers. Figure 1*c* shows the spectrum of H_2O 316.8−0.1, the most intense source found in that class during our survey. Another interesting member of that class of sources is H_2O 291.7−0.7 (Fig. 1*d*). At least one strong emission line can be observed at −32.8 km s^{-1}, with an intensity of 159 Jy superimposed on a 1.8-K continuum level. That source, and H_2O 267.9−1.1 (identified with RCW38), are known to be associated with compact protostellar

Fig. 1 *a*, H_2O 345.5+0.3, the strongest source found in association with OH main-line emission (spectrum is average of three 6-min runs); *b*, spectrum of H_2O 0.55–0.85 (RCW142), (integration time, 6 min); *c*, H_2O 216.8–0.1, the strongest source not associated with OH main-line emission (spectrum is average of two 6-min runs); *d*, H_2O 291.3–0.7, associated with the extended H II region RCW57, which contributes the 1.8-K continuum level (spectrum is the average of four 6-min runs).

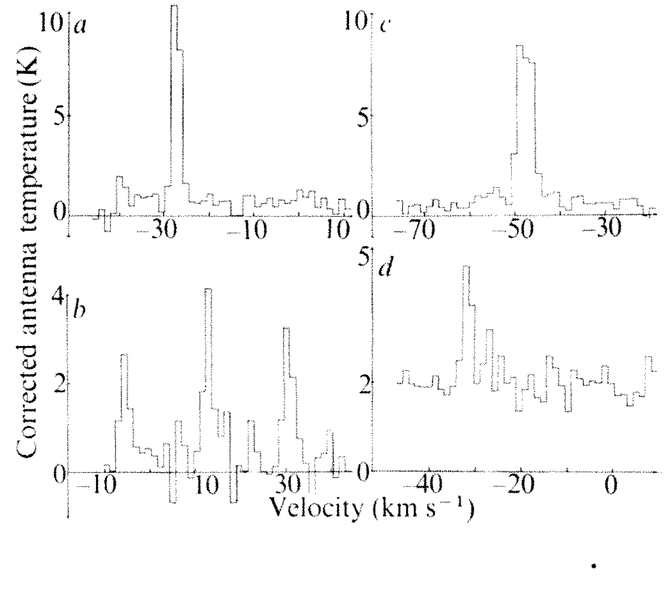

Table 1 New H_2O celestial masers in Southern Hemisphere H II regions

Galactic coordinates	H_2O line peak intensity in Jy (November 1975)	Radial velocity at peak intensity (km s^{-1} in relation to LSR)	Reference for p positions	Comments
Sources associated with OH main-line emission				
0.55−0.85	128	+11.6	11	RCW142. One 6-min run, centred at +13 km s^{-1}.
330.0−0.4	45	−67.2	9	Three 6-min runs, centred at −60 km s^{-1}.
333.2−0.1	59	−84.6	10	Two 6-min runs, centred at −90 km s^{-1}.
338.9+0.6	127	−76.3	9	Two 6-min runs, centred at −60 km s^{-1}, and −75 km s^{-1}.
345.5+0.3	359	−28.1	9	Three 6 min runs, centred at −20 km s^{-1}. See Fig. 1.
347.6+0.2	49	−83.8	9	Two 6-min runs, centred at −100 km s^{-1}. Another possible weaker line at −94.6 km s^{-1}.
Sources at regions that do not exhibit OH main-line emission				
267.9−1.1	171	− 6.1	8	RCW38. One 6-min run, centred at +2 km s^{-1}.
291.3−0.7	159	−32.8	8	Four 6-min runs, centred at −22 km s^{-1}. See Fig. 1*d*.
305.3+0.2	79	−42.7	8	Three 6-min runs, centred at −40 km s^{-1}. Another possible 53 Jy, line at −40 km s^{-1}.
316.8−0.1	290	−48.6	8	Two 6-min runs, centred at −50 km s^{-1}. See Fig. 1*c*.
326.7−0.6	70	−41.3	8	Three 6-min runs, centred at −20 km s^{-1}; two 6-min runs, centred at +5.2 km s^{-1}. Other lines at −7.0 km s^{-1} (60 Jy), −13 km s^{-1} (66 Jy), and −62 km s^{-1} (61 Jy).
332.7−0.6	58	−47.3	8	Two 6-min runs, centred at −50 km s^{-1}.
348.7−1.0	155	−12.7	8	Two 6-min runs, centred at −10 km s^{-1}. Another possbile 65 Jy line at −31.6 km s^{-1}.

infrared objects[14]. Another source found, tentatively designated as H_2O 333.0—0.4, and showing a 64-Jy line at −51.3 km s^{-1}, is possibly a confusion with a known nearby water vapour source, H_2O 333.2−0.5, which has OH main-line emission[7].

Our results are preliminary, and a detailed report is in preparation. We acknowledge technical assistance by V. Saraceni, R. Hadano and M. Shimabukuro, at Itapetinga, and by R. Erickson, N. Lariviere and S. Murray at the Haystack Observatory, Westford, Massachusetts. We also acknowledge the assistance of the Department of Physics of Campinas State University, Campinas, Brazil, for the liquefaction of helium and associated technical help needed to support the maser receiver. This research programme received support from Brazilian research agency Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) and in the US from the National Science Foundation (NSF), through a bilateral scientific agreement.

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Received January 9; accepted February 6, 1976.

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Observations of Cyg X-1 from Aryabhata

ARYABHATA, India's first artificial satellite, which was launched into a near circular orbit of 600 km altitude at an inclination of 51° on April 19, 1975, carried instruments to investigate celestial X rays in the energy range 2.5–155 keV. We report here observations of Cyg X-1 made in the range 2.5–18.75 keV, during the second orbit of the satellite, centred around 108 d 9 h 17 min 40 s UT, just before the source made a major increase in its intensity. We found a hardening in the spectrum.

We investigated this energy range with a proportional counter telescope filled with a mixture of Ar, CO_2 and He in the pressure ratio of 90:9.5:0.5 to a total pressure of 1 atm. The counter, which had a 2.5 0.0025-inch Be entrance window and an effective area of 15.4 cm^2, was fitted with cylindrical aluminium collimators for restricting the field of view to 12.5° FWHM. This telescope was mounted with its viewing direction along the spin axis of the satellite so that it could be oriented stably and operated in the pointed mode. The sighting on Cyg X-1 was made possible before the spacecraft was spin stabilised and during the time when it was performing an extremely slow spin around an axis different from the actual spin axis.

The counting rates were monitored in four energy channels: 2.5–5.4 keV, 5.4–9.8 keV, 9.8–13.8 keV and 13.8–18.75 keV, thus enabling the spectral determination of the source. Background rates were evaluated from the study of the counting rate profile as a function of time as well as their known dependence on the geographical latitude of the satellite. The attitude of the X-ray detector in space was computed using the outputs from magnetometers and Sun sensors mounted on the satellite. The excess rates corresponding to Cyg X-1 were corrected for the offset angle of the telescopic axis with respect to the

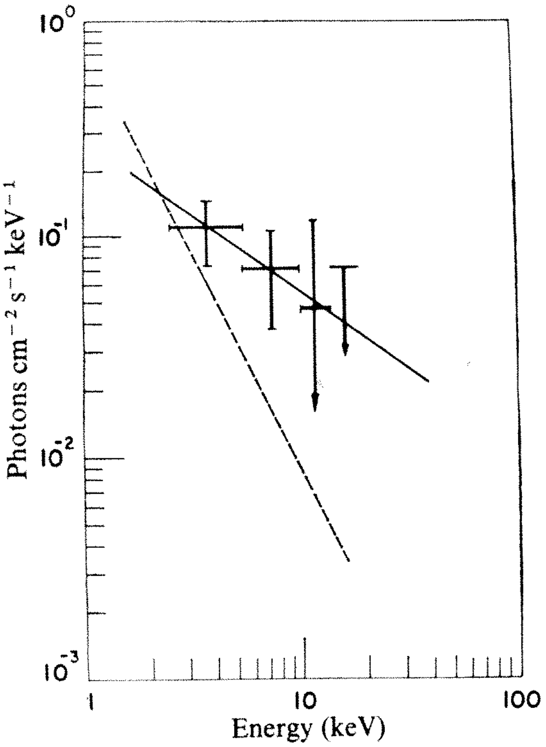

Fig. 1 The spectrum of Cyg X-1, as observed on 108 d 9 h 17 min 40 s UT (April 19, 1975), before the intensity increase. The dotted line gives the spectrum observed by Ariel V on May 13, 1975.

source direction and for the efficiency of the counter as a function of energy. The photon spectral distribution so derived is shown in Fig. 1. The spectrum shows a power law dependence with an index of ~ 0.7±0.2 in the energy interval of 2.5–13.8 keV, the estimated intensity being 0.84±0.3 photon cm^{-2} s^{-1} at the time of observation.

Cyg X-1 underwent a major upward transition in its intensity during late April 1975, as revealed by the detectors on the Ariel and ANS satellite[1-3]. This intensity transition seems to be the reverse of the one observed by Uhuru[4] in April 1971. A distinct feature of these intensity transitions is the accompanying spectral change[3], with the spectral exponent changing from the value of 1.6 observed during the normal state to 2.2 in the 'high' state. The all-sky camera on Ariel V (ref. 2) seems to place the occurrence of the intensity transition around the latter half of April 22, 1975 based on long term intensity monitoring fo this source in the 3–6-keV energy range from November 1974 to May 1975. The results presented here complement the results of the all-sky camera by providing definitive spectral information, for the first time, just before the transition. Thus, together with Ariel observations, it is now possible to examine the physical state of this X-ray source just before and just after this low–high transition. Whereas the intensity from Cyg X-1 registered by our detector in the 3–6 keV range (~ 0.3±0.1 photon cm^{-2} s^{-1}) is comparable with that of all-sky camera (~ 0.4±0.1 photon cm^{-2} s^{-1}), the spectrum observed by us is harder than the typical one for this source, even in its normal state. For purposes of comparison, the spectral nature of the source after the upward intensity transition as observed by Ariel V (ref. 3) is also shown in Fig. 1. It is, however, to be noted that the spectral hardening of this type with time scales of the order of minutes has been reported[1] previously also for Cyg X-1 in its normal state. Whether such spectral hardening forms a prelude to the transition of the source or is a property of its short term fluctuations, not necessarily related to the transition, needs further investigation.

Cyg X-1 is an interesting and complex X-ray source, probably in a binary system, where X-ray emission takes place during the mass transfer from the OB supergiant to a compact companion. This source is known to exhibit intensity variations with time scales of the order of milliseconds, minutes and even days[5,6]. Flare-like enhancements[7], sudden intensity transitions[4] and intensity dips around superior conjunction of the spectroscopic binary have also been observed.

Attempts have been made to understand the intensity transition in Cyg X-1 in terms of the variable mass accretion from the OB supergiant by the compact companion[8] and the possible instabilities in the accretion disk[9]. Dips in intensity observed in association with the superior conjunction of the binary[10], resulting in spectral hardening, can arise from discrete absorption features such as gas streams of localised character[11]. Such features can also explain short term hardening of the spectrum of the type reported here. A single self-consistent explanation that can account for all the emission characteristics of Cyg X-1 has, however, yet to emerge.

This work was supported by the Department of Space, Government of India. The launching of the Indian satellite Aryabhata was made possible through joint cooperation between the Indian Space Research Organisation and the Academy of Sciences, USSR.

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Received December 18, 1975; accepted January 30, 1976.

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Intrusions and double-diffusive convection

A STRATIFIED fluid layer in which two components contribute to the vertical density distribution need not be stable even though the net density decreases upwards. If one of the components is unstably distributed, then molecular diffusion can release its potential energy—a phenomenon known as double-diffusion convection[1]. Considerable progress has been made over the past 10 years in the study of double-diffusive convection in situations where the properties vary in one direction (vertically) only. In the ocean, horizontal advection is nearly always important and its neglect in the previous laboratory experiments makes it difficult to apply the results in a wider context. A first step in the study of two-dimensional effects in double-diffusive convection was made by Turner and Chen[2], and here I follow up one of their suggestions in detail in the hope that it may help to understand the layering which occurs when an outflow intrudes into an ambient fluid with the same density but different component concentrations[3].

Convection (when driven by double-diffusive processes) occurs in well mixed layers separated by comparatively thin regions of high density gradients, called interfaces. The interface can take either of two forms depending on whether the potential energy, which drives the motion, is provided by the component of higher or lower diffusivity. In the former case a 'diffusive' interface is formed; in the latter case a 'finger' interface[1]. Layering that can be asso-

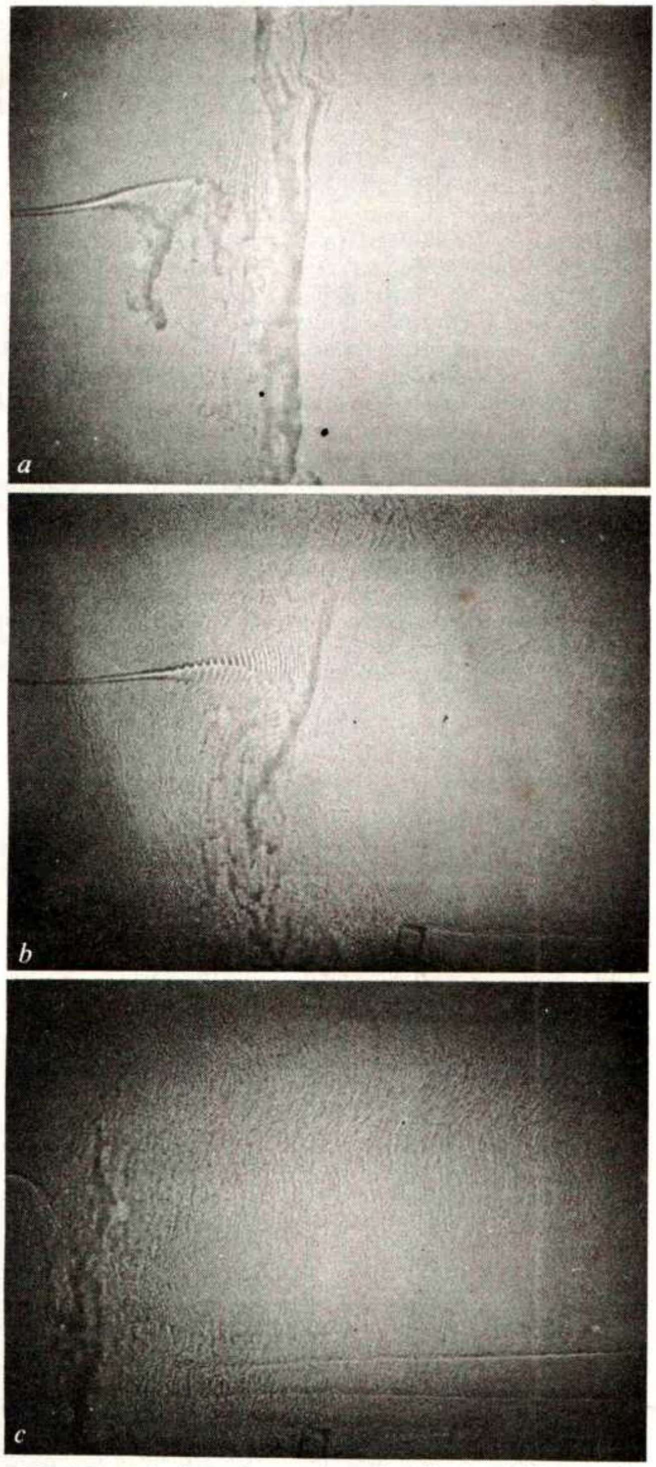

Fig. 1 The behaviour of a sugar intrusion (density, 1.180 g cm^{-3}) into a salt solution (same density): *a*, after 30 s; *b*, after 1 h; *c*, after 2 h.

ciated with both types of double-diffusive processes has now been observed in the ocean.

The experiments were performed in a transparent tank 98 cm long, 29 cm deep and 15 cm wide. The flow was observed using the shadowgraph technique. A small source (internal diameter 1 mm) was located at one end of the tank at mid-depth and the fluid was released in an approximately horizontal direction. Some preliminary experiments were performed in which a salt solution was released into an homogeneous salt solution of the same density (1.180 g cm^{-3}). The flow rate was adjusted so that the motion remained laminar. The released fluid was found to propagate smoothly across the tank with very little mixing. The same flow rate was used in all subsequent experiments.

Two types of experiment were performed, and because of their similarity only one is described in detail here. In the first type of experiment the tank was filled with an initially homogeneous salt solution with a density of 1.180 g cm^{-3}, and a sugar solution was released. The density of the sugar solution was adjusted until it too was 1.180 g cm^{-3}, and then minute adjustments were made so that when released into the salt solution it initially moved horizontally. The sugar solution was left flowing for periods of up to 3 h.

Figure 1*a* shows the behaviour of the plume after 30 s. The downward motion on the left hand side of the main vertical motions was caused by unsteadiness in turning on the intrusion. The plume becomes denser (because the diffusivity of salt is greater than that of sugar) and sinks. The immediate surroundings become less dense as they lose more salt than they gain sugar, so they rise. Thus vertical motions are generated in both upward and downward directions, thus stratifying the tank. Figure 1*b* was taken after 1 h. A diffusive interface can be seen near the bottom of the tank, and fingers can be seen growing from the top of the tank. The downward plume comprises sugar with a little salt, and as it is denser than the ambient fluid it spreads out along the base of the tank. Thus, near the base the salt gradient is destabilising, and as it is the component with greater diffusivity, a diffusive interface is formed.

The upward plume carries a reduced amount of salt (in comparison with the ambient concentration) and a little sugar. This plume spreads out on the surface. The sugar has a destabilising effect, and fingers are formed near the surface. The regular vertical striations which can be seen on the upper edge of the plume near the bending point were stationary and had the appearance of crests in a standing wave pattern. They are also evident in Figs 1*a* and 2*a*. Figure 1*c* was taken after 2 h and shows the final stage in the experiment. There is now extensive fingering in the upper half of the tank and several diffusive interfaces in the lower half. These double-diffusive processes will further stratify the fluid.

Thus, strong vertical motions are generated, carrying excess sugar to the surface ('excess' in this context means a more concentrated solution than that immediately below the surface), and reduced salt to the base of the tank. These concentration profiles provide available sources of potential energy which drive the double-diffusive processes. The vertical motions stratify the fluid and the double-diffusive processes further stratify it.

In the second type of experiment a salt solution, with a density of 1.180 g cm^{-3}, was released into a sugar solution of the same density. Again, strong vertical motions were generated; however, this time the released solution rose carrying an excess of salt to the surface, and the downward plume carried a reduced sugar concentration to the base. These concentration profiles produced diffusive interfaces at the surface of the tank, and fingers at its base. These features are illustrated in Fig. 2*a* which was taken after 1 min, and in Fig. 2*b* which was taken 2 min after the experiment ended (about 2 h after Fig. 2*a*).

The Mediterranean outflow consists of hot salty water which intrudes at its own density level into the cooler, less salty Atlantic Ocean, forming a salinity maximum at 1,200 m. Layering consistent with the finger process has been found beneath the Mediterranean outflow[3] and layering stratified in the opposite sense has been found above it. Williams[4], using a sophisticated optical system has photographed salt fingers below the Mediterranean outflow, thus increasing confidence in the double-diffusive explanation of the layering. In the Mediterranean outflow the sites for the initiation of double-diffusive convection occur at the upper and lower interfaces of the intruding wedge of hot salty water. At the upper boundary, heat is the destabilising

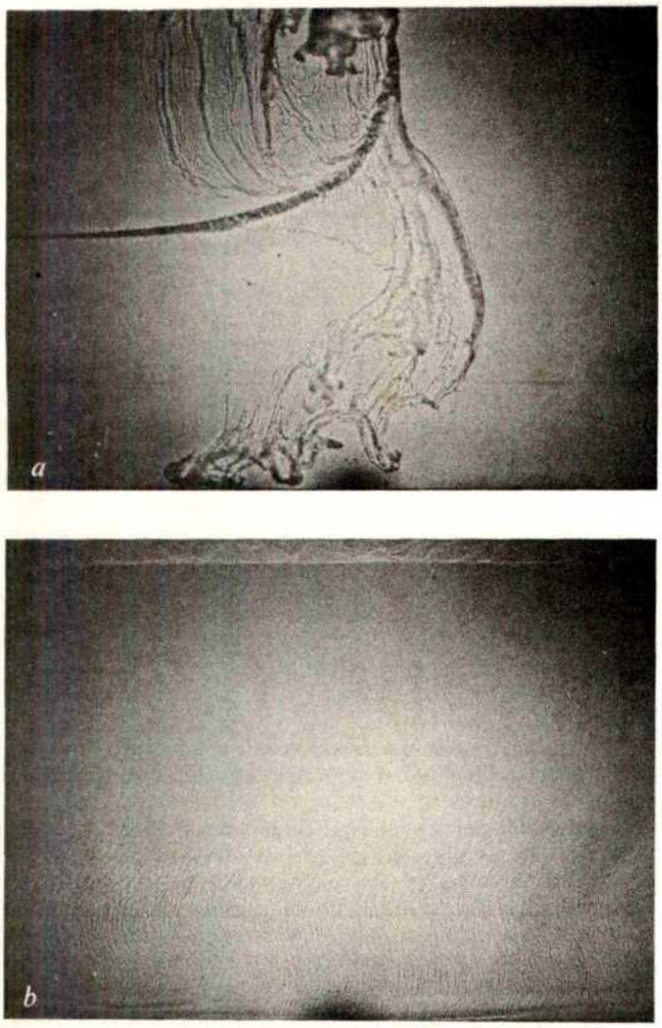

Fig. 2 The behaviour of a salt intrusion (density, 1.180 g cm^{-3}) into a sugar solution (same density): *a*, after 1 min; *b*, 2 min after end of experiment.

component giving diffusive interfaces, and at the lower boundary, salt is the destabilising component giving fingers. In the laboratory, the vertical plumes produce the destabilising gradients at the upper and lower boundaries of the tank. The oceanic situation could be modelled more accurately by using a two-component solution (initially homogeneous, then with both gradients stabilising, and finally with either gradient destabilising) and a two-component intrusion with different concentrations but the same density as at the release depth.

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Received November 19, 1975; accepted February 6, 1976.

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New Zealand temperatures since 1300 AD

THE period 1900–1935 was the coldest in New Zealand in recorded history. A climatic amelioration was experienced throughout the whole country between 1935 and 1970, with temperatures climbing by 1 °C over those years[1]. It is interesting to examine recent glacial behaviour in the light of these facts.

New Zealand west coast glaciers, such as the Fox and Franz Josef glaciers, are fast moving because of their steep gradients; they are also nourished by high precipitation rates. As a result, they respond rapidly to climatic fluctuations; however, glaciers are complex systems, so conclusions regarding climatic events must be treated cautiously. The west coast glaciers examined here descend to low altitudes (300 m) and flow rapidly, so the position of their terminal faces can be used as climatic indicators.

The recent glacial retreat shows a good relationship with running means of Christchurch mean annual temperatures, allowing for a few years' lag in the response time of the glacier (Fig. 1). Hokitika precipitation has a high correlation with Franz Josef precipitation[2]. There seems, however, to be little correlation with recent glacial movements. It seems that the west coast glaciers are not in equilibrium with the present warmer climatic regime and that they are responding by retreating rapidly. The better agreement with temperatures validates the use here of the position of the terminal face and past terminal moraines as indicators of palaeotemperatures.

A glacial advance will usually obliterate all terminal moraines in its path. The most advanced terminal moraines of the Franz Josef and Fox glaciers were laid down around 1600 AD. An advance of a greater magnitude occurred at the Franz Josef glacier at 4,730 yr BP. Since 1600 AD, a minor retreat occurred up to 1800, after which the rate of retreat was moderate; since 1934 it has been rapid (Fig. 2 and ref. 3).

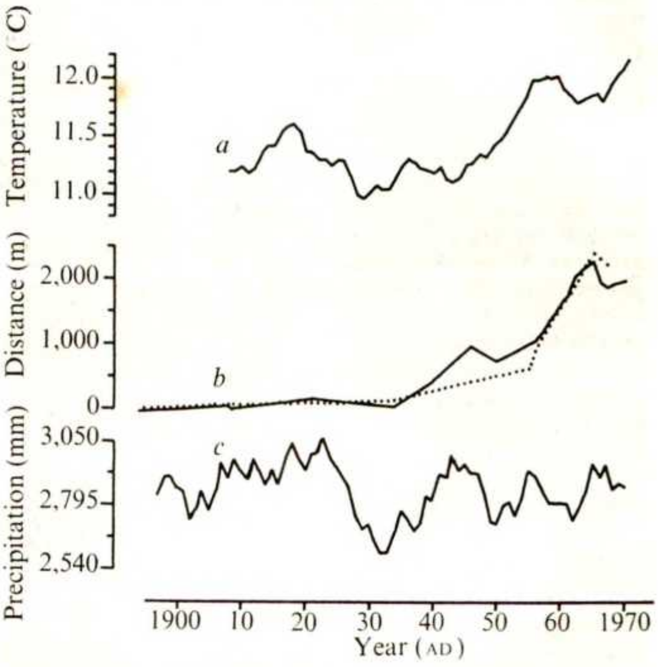

Fig. 1 Comparison of recent New Zealand west coast glacial behaviour with climatic parameters: *a*, seven-year running means of mean annual temperature at Christchurch; *b*, fluctuations of the terminal faces of the two west coast glaciers (—, Franz Josef; · · · Fox); *c*, seven-year running means of Hokitika annual precipitation.

During the period 1600–1800, the glaciers were much more advanced than at present and were also apparently in equilibrium, which indicates a climate much cooler than at present. The shrinkage of the terminal faces since 1800 suggests a climatic amelioration, and the recent rapid wasting correlates well with the recent rapid climatic warming experienced in New Zealand.

Evidence of temperature changes before 1600 AD have to be gleaned from other palaeoindicators. Wardle notes a regeneration gap occurring in New Zealand gymnosperms between 1300 and 1900 AD, with the gap being most accentuated between 1600 and 1800 AD (ref. 4). Holloway, however, postulated a climatic deterioration around 1300 to explain anomalies

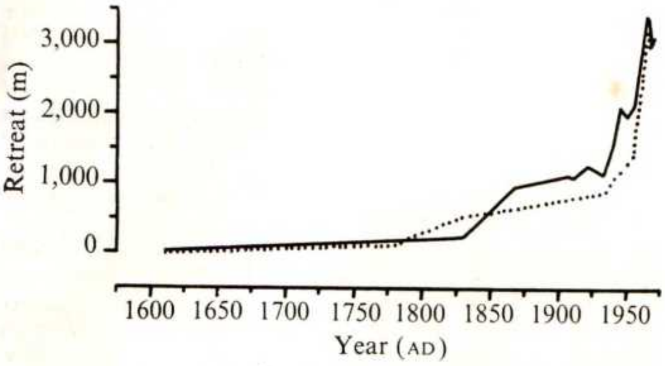

Fig. 2 Retreat of the terminal faces of the Franz Josef (—) and Fox (. . .) glaciers from their most advanced positions in the last millennium.

in the age structure of certain indigenous conifer stands on the South Island[5]. Molloy has suggested that cultural factors such as fire could have contributed to these anomalies[6]. Both Wardle and Holloway have examined forests in areas of dependable precipitation. With the coincidence between these ecological indicators and west coast glacial chronology[7], non-climatic explanations seem untenable. Thus, it seems that New Zealand experienced a climatic deterioration starting around 1300 AD. Being most severe between 1600 and 1800, it correlates well with the Little Ice Age of the Northern Hemisphere[8]. Since 1800, a slow warming has occurred, culminating in the rapid, 1 °C warming of the past 40 yr.

New Zealand palaeotemperatures seem to be similar to those of Northern Hemisphere[9] areas except during the present century, but some doubt must remain since dating was not accurate before temperature records were kept. Divergence between Northern Hemisphere and Southern Hemisphere temperatures may be a phenomenon of the past 50 yr. This assumes that New Zealand trends mimic those covering a wider area of the Southern Hemisphere. Evidence from south-eastern Australia (M. J. Coughlan, unpublished) Scott Base in Antarctica, Campbell Island, and Orcadas Island near South America at latitude 60°45′S, all demonstrate a temperature rise beginning in the 1940s.

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Hydrodynamic instabilities in a lead acid battery

WE demonstrate here the feasibility of the observations of hydrodynamic instabilities in a lead acid battery during the discharge process. We use a system comprising two horizontal electrodes immersed in a transparent cell. The electrodes contain in the charged state porous PbO_2, the 'positive' electrode, and porous Pb, the 'negative' electrode. The electrodes were prepared to commercial standards using common industrial processes.

Hydrodynamic patterns in a transparent medium, with accompanying gradients of specific gravity, can be easily followed using a coherent and monochromatic light source. Specific gravity gradients in liquids are usually accompanied by parallel gradients in the index of refraction. Therefore, phase differences induced in the coherent light on passing through the medium are observed as an amplitude pattern when viewed out of the image plane. This is an interference phenomenon occurring between light waves traversing different optical paths in the medium.

The experiment was performed as follows. Light from a 5,145-Å Argon laser passes through a spatial filter and is then collimated on the $Pb–PbO_2$ transparent cell. The hydrodynamic pattern which occurs between two horizontal electrodes is projected on to a screen and recorded on photographic film. A few examples are shown in Fig. 1. The occurrence of such patterns above a certain current density during the discharge process is predictable from stability considerations. The chemical reaction during the discharge process is

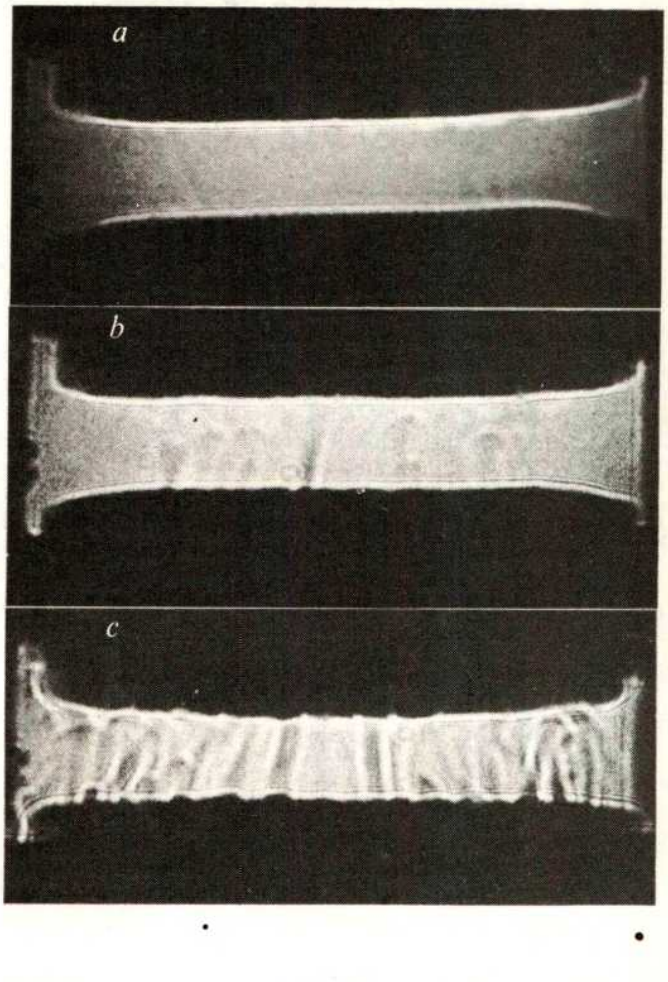
'Positive' electrode: $PbO_2 + 2e + SO_4^{2-} + 4H^+ \rightarrow PbSO_4 + 2H_2O$

(Similar reactions occur with HSO_4^- ions)

'Negative' electrode: $Pb + SO_4^{2-} \rightarrow PbSO_4 + 2e$

The electrodes therefore serve as a sink for the acid and the 'positive' electrode is a source of water. In a horizontal set-up of the electrodes at low enough currents, a steady-state profile of acid concentration, and, therefore, of density will be established. This profile is shown schematically in Fig. 2. To a first approximation a low acid concentration is established at the

Fig. 1 Hydrodynamic patterns between electrodes: *a*, $i = 0$ A cm^{-2}; *b*, $i = 6 \times 10^{-3}$ A cm^{-2}; *c*, $i = 30 \times 10^{-3}$ A cm^{-2}. All experiments performed at 25 °C.



electrodes' surfaces, and a maximum concentration is established somewhere in the electrode gap.

This distribution of density in the y direction can be sustained up to a certain gradient for a given cell geometry. The maximum current density which develops at the maximal gradient is easily calculated from the expression

$$i_{\max} = 2eD(\nabla C)_{\max}$$

where e is the elementary charge and D is the diffusion coefficient for H_2SO_4 aqueous solution. Thus, for example, with an electrode gap of 1 cm; ($y = 1$ cm) and a 1.2 g cm^{-3} acid (27% by weight), we have

$$(\nabla C)_{\max} \simeq \frac{\text{Number of carriers}}{y_0/2}$$

$$= \frac{(\text{Weight \% } H_2SO_4) \times (\text{Avogadro number})}{(\text{Molecular weight of } H_2SO_4) \times (y_0/2)}$$

$$= 3.3 \times 10^{21} \text{ carriers per cm}^2$$

$$D_{\rho=1.2} = 1.34 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$$

Therefore

$$i_{\max} = 14 \times 10^{-3} \text{ A cm}^{-2}$$

This estimation of $i_{\max}$ is really an upper limit since the y gradient of water generates a flow opposing the above current.

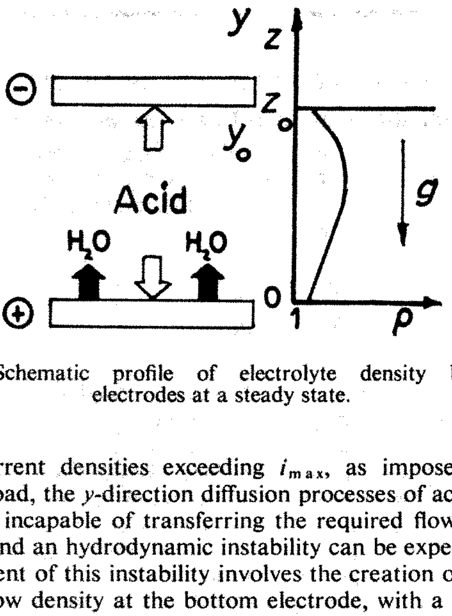


Fig. 2 Schematic profile of electrolyte density between electrodes at a steady state.

For current densities exceeding $i_{\max}$, as imposed by an external load, the y-direction diffusion processes of acid and of water are incapable of transferring the required flow rates of carriers, and an hydrodynamic instability can be expected. The development of this instability involves the creation of a water layer of low density at the bottom electrode, with a region of high density on top of it. In a gravitational field this inverted distribution of densities is discharged into patterns of ascending water and descending acid (Fig. 1).

Experimentally, an instability occurs at ~5 mA cm^{-2} and a pattern of pillars emanates from the bottom positive electrode towards the top negative electrode. The pillar pattern is of quite a regular geometrical nature and is preserved up to quite high current densities of ~0.5 A cm^{-2}. Only above that limit does the pattern break so that turbulent phenomena are observed.

We believe that the hydrodynamic instability described, and its observation, may provide a useful tool for the understanding of the performance of Pb–PbO_2 batteries at high discharge currents. The system may serve as a model to elucidate the limits imposed by hydrodynamic phenomena on the performance of the lead acid battery. It should be pointed out that the phenomena presented occur in the whole range of technically feasible discharge rates.

B.G. were supported by a Katzir–Katchalsky fellowship.

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Received November 4, 1975; accepted January 28, 1976.

Tungsten trioxide as a photoanode for a photoelectrochemical cell (PEC)

THE PEC[1] has acquired popularity among researchers in the past years, mainly because of its possible use in solar energy conversion[2,3]. The major problem at present is to find a semiconductor electrode with an optical band gap (written as just band gap below) small enough to allow it to absorb a reasonably large portion of the solar spectrum, and at the same time being stable against photocorrosion. We here report that WO_3 meets these criteria although it is not very efficient at solar wavelengths.

Until now, the only material which was known to be stable as a photoanode absorbing in some region of the solar spectrum, is TiO_2, with a band gap $E_G = 3.05$ eV corresponding to a wavelength of 408 nm—on the border of the visible and ultraviolet. In the search for other stable photoanodes of smaller E_G, one criterion, which we used, is that one of the chemical elements making up the semiconductor material should be capable of reversibly changing its valence state to accommodate a hole without decomposing the semiconductor (as $Ti^{3+} \rightarrow Ti^{4+}$ in non-stoichiometric TiO_2) instead of having only one possible stable valence state in the semiconductor (as Cd^{2+} in CdS or Zn^{2+} in ZnO, both of which are decomposed by holes). WO_3, an n-type semiconductor with $E_G = 2.8$ eV, satisfies this criterion, and was selected for trial as a photoanode in a PEC.

WO_3 electrodes were prepared either by heating tungsten metal to form a layer of yellow WO_3, or by spraying ammonium tungstate on conducting glass (a gold layer on glass) and heating at ~ 500 °C to decompose the tungstate. Higher photocurrents were obtained from electrodes formed by heating the metal, but apart from the spectral response of the photocurrent, the general properties were much the same for both types of electrodes. X-ray powder diffraction of the samples showed the composition of the layers to be WO_3 with traces of lower oxides. The WO_3 electrode was connected to a platinised platinum oxygen counter electrode, and both electrodes were immersed in 1 N H_2SO_4 in a quartz cell.

In AM1 sunlight (AM1 = airmass 1, which is equivalent to directly overhead sunlight with energy of ~ 1 kW m^{-2}) photocurrents of ~ 0.2 mA cm^{-2} were obtained with WO_3 on tungsten and typically, one quarter of this for WO_3 on conducting glass. (Dark currents were $< 10^{-4}$ mA cm^{-2}.) The open circuit potential between the two electrodes increased from ~ 280 mV in the dark to 400 mV on illumination. Figure 1 shows the difference in currents obtained, in a potentiostatic experiment, between a WO_3 electrode under illumination and in the dark. The start of oxygen evolution shifted from +2.0 V (an overpotential of ~ 0.8 V) in the dark to +0.6 V (an underpotential of ~ 0.6 V) on illumination—a shift of 1.4 V.

That the photocurrent came from oxidation of water, and not photocorrosion of the WO_3 layer, is evident from two observations. First, after continuous passage of 42 C of electricity, which, if 100 per cent corrosion occurred, would correspond

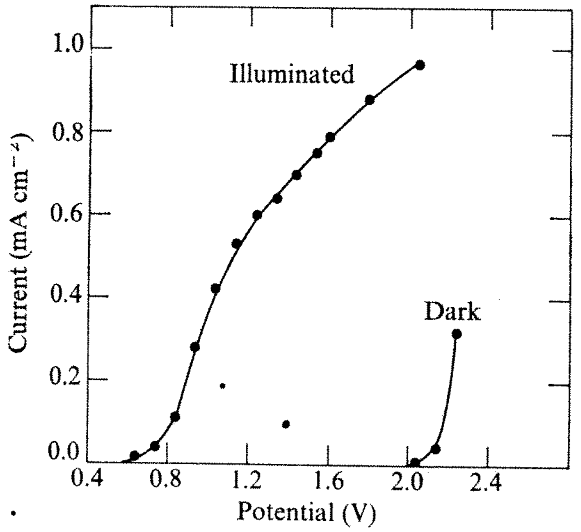
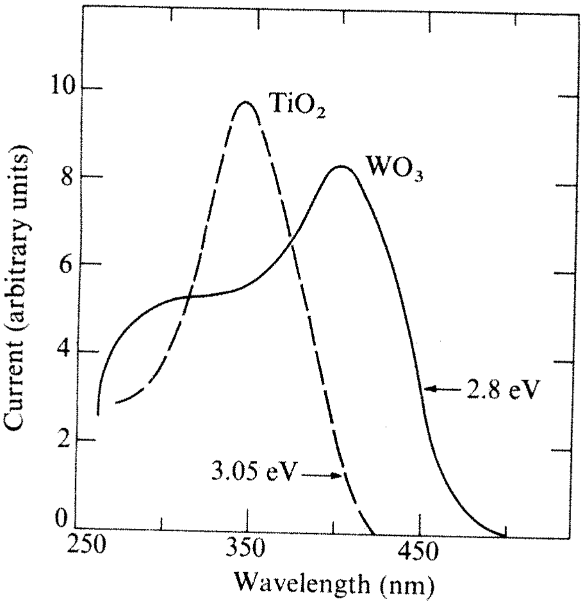


Fig. 1 Current–potential curves for a WO_3–tungsten photoanode in the dark and on illumination with light approximating to sunlight. (Measurements were made using a standard calomel electrode (SCE) as reference, and the potentials shown are adjusted to the standard hydrogen electrode (SHE) scale.)

to the dissolution of 50 mg of WO_3 (assuming the hypothetical dissolution occurred by a two-electron transfer), there was no change in current, and no change in the weight of the electrode to < 0.5 mg. Second, if a strong source of light was used, so that a larger current ($\gtrsim 1$ mA cm^{-2}), was obtained, gas evolution from the electrode was clearly evident.

Besides stability, the next important property of a suitable photoanode for solar energy conversion is the ability to absorb well in the visible spectrum. Theoretically, the absorption edge for WO_3 should be shifted ~ 40 nm to the red, compared with TiO_2. The spectral response of the WO_3 electrode is compared in Fig. 2 with that for a polycrystalline TiO_2 electrode prepared by heating titanium metal in oxygen, followed by heating in hydrogen.

Fig. 2 The spectral response of the photocurrent for an electrode of WO_3 on conducting glass compared with a polycrystalline TiO_2 electrode. The positions of the wavelengths corresponding to the band gaps are arrowed (3.05 eV $\simeq$ 410 nm; 2.8 eV $\simeq$ 450 nm). The current at the peak for TiO_2 is actually considerably higher than the corresponding current for WO_3—they are shown nearly equal for purposes of clarity. (The total short-circuit current in sunlight for the TiO_2 electrode is $\sim$0.8 mA cm^{-2} compared with $\sim$0.2 mA cm^{-2} for a WO_2 electrode.)



The absorption edge for WO_3 was, in fact, shifted by ~ 50 nm. Figure 2 shows typical results obtained with WO_3 on glass. For WO_3 on tungsten, instead of the current decreasing with decreasing wavelength after the peak at 400 nm, there is a broad plateau, or slow increase, between 400 and 360 mn, followed by a sharper increase to a peak at ~ 300 nm—much like the mirror image of the curve shown. It should be stressed that the part of the curve in the visible region, 400–500 nm, is similar for both types of electrode. The difference in the ultraviolet may conceivably arise from a difference in crystallinity of the two types of WO_3. In other materials such as Si, there is a difference in spectral response between the amorphous and the crystalline states[2].

The important point arising from these preliminary results is not to suggest that WO_3 may be a serious candidate for an economical solar cell (the absorption still occurs over too small a part of the solar spectrum) but rather to show that stability against photocorrosion is not unique to TiO_2 (or other semiconductors with an even larger gap, such as Ta_2O_5, which may also be stable, but do not absorb in the solar spectrum), and that, where one more stable compound with a smaller band gap has been found, there may be others with even smaller gaps.

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Received December 19, 1975; accepted February 12, 1976.

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Effect of surface roughness on rolling friction and adhesion between elastic solids

THEORETICAL analyses of the influence of surface roughness on the adhesion of elastic solids[1,2] suggest that the introduction of roughness should reduce the adhesion to an extent governed by an adhesion parameter

$$\alpha = \left(\frac{4\sigma}{3}\right)\left[\frac{4E}{3\pi\beta^{\frac{1}{2}}\gamma}\right]^{2/3}$$

where α is the distribution of asperity heights (assumed to be Gaussian), β the mean radius of curvature of the asperities and γ is the net change in surface energy per unit area on the formation of adhesive contact. Experiment[2] bears out the theory. Here we report experiments on rolling, when we found α again to be a useful parameter. At low roughnesses, however, we found a surprising new result, that the adhesion could be greater than for smooth surfaces, and we propose an explanation for this.

Roberts and Thomas[3] and, independently, Kendall[4] had shown that in rolling friction the adhesion term may sometimes be dominant. In these circumstances the rolling friction provides a direct measure of the work required to peel apart and re-adhere the interface. This depends on the velocity, and may be thought of as the product of the surface energy and a visco-elastic term.

For smooth surfaces, the force required to separate a sphere from a flat had been shown by Johnson *et al.*[5] to be

$$P = -(3/2)\gamma\pi R$$

The reduction in the force with increasing roughness comes from the high asperities prising the surfaces apart and reducing the true area of contact. It is therefore to be expected that

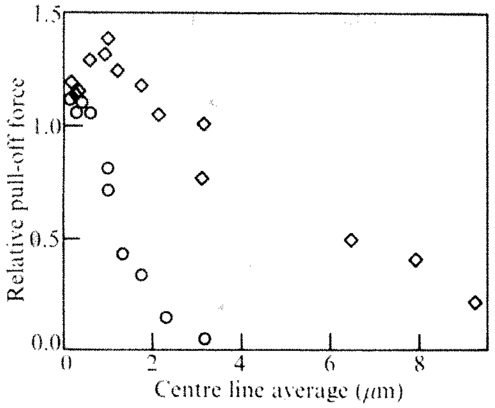

Fig. 1 Relative pull-off force for a smooth rubber surface in contact with a Perspex surface as a function of the roughness (centre line average) of the Perspex, using a crossed cylinders configuration. The pull-off force is normalised to be unity for a nominally smooth Perspex surface. ○, RTV 602 ($E = 4.9 \times 10^6$ Pa); ◇, Dow Corning XF—13-523 ($E = 6.3 \times 10^4$ Pa). In both cases the pull-off force for a smooth surface indicated a surface energy of $\gamma \simeq 100$ mJ m^{-2}. For a Gaussian distribution of asperity heights, the centre line average is a good measure of σ.

adhesion measured by rolling experiments will be affected by surface roughness in a similar way to the pull-off measurements.

We measured the adhesion during rolling at various speeds, for two rubbers, over a range of roughnesses. Perspex cylinders were roughened by blasting with fine particles. The surfaces were characterised by profilometry measurements, with data processing using established techniques[6] and thus β and σ were deduced. These cylinders were rolled down an inclined plane surface of silicone rubber and the time taken to roll 140 mm was measured electronically. The adhesion energy was determined from the weight of the cylinder and the angle of inclination. These measurements were much more reproducible (±2%) than those of Fuller and Tabor, partly because the adhesion was averaged over the whole of the surface of the cylinder and also because the time dependence was precisely specified. The greatest errors were in the surface characterisation (±10%), also depending on sampling lengths.

For the purpose of comparison with previous work the pull-off force for the various surfaces was measured using a crossed cylinders configuration. The results are shown in Fig. 1. Figure 2 shows the results for the rolling experiment, and a comparison of these two graphs shows that the effects of changing roughness and modulus are very similar in the pull-off and rolling experiments. In the case of the rolling experiments, it seems possible (except for the peak discussed below) to separate out the viscoelastic time dependence and the effect of roughness, since the normalised points for different rolling speeds fit closely over one another when the normalised adhesion $\lesssim 1$.

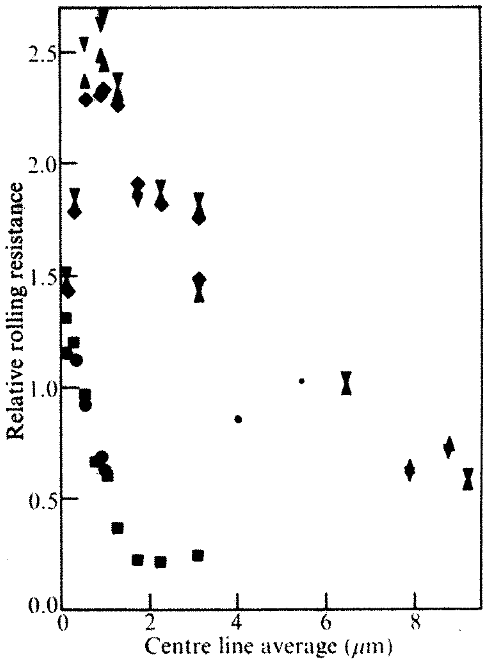

Fig. 2 Relative rolling resistance for Perspex cylinders (radius 5 mm, length 10 mm) rolling down an inclined smooth rubber surface at the following speeds (mm s^{-1}). RTV 602: ●, 2; ■, 4; Dow Corning XF—13-523; ◆, 0.5; ▲, 1; ▼, 2. The adhesion energy is the net work required for a complete cycle of adhering together and then peeling apart unit area of interface, and is deduced directly from the rolling resistance. It is markedly velocity dependent[3,4,7]. The absolute values for nominally smooth surfaces, to which the above points are normalised, are respectively (mJ m^{-2}): 600; 970; 253; 384; 612. Dusting the rubber surface with talcum powder to remove the adhesion reduced the relative rolling resistance to a very small value, indicating that bulk ploughing hysteretic losses were almost negligible in these experiments.

A new feature became apparent during these studies, however, which had previously been neither predicted nor observed. For very small roughnesses, the adhesion may be considerably

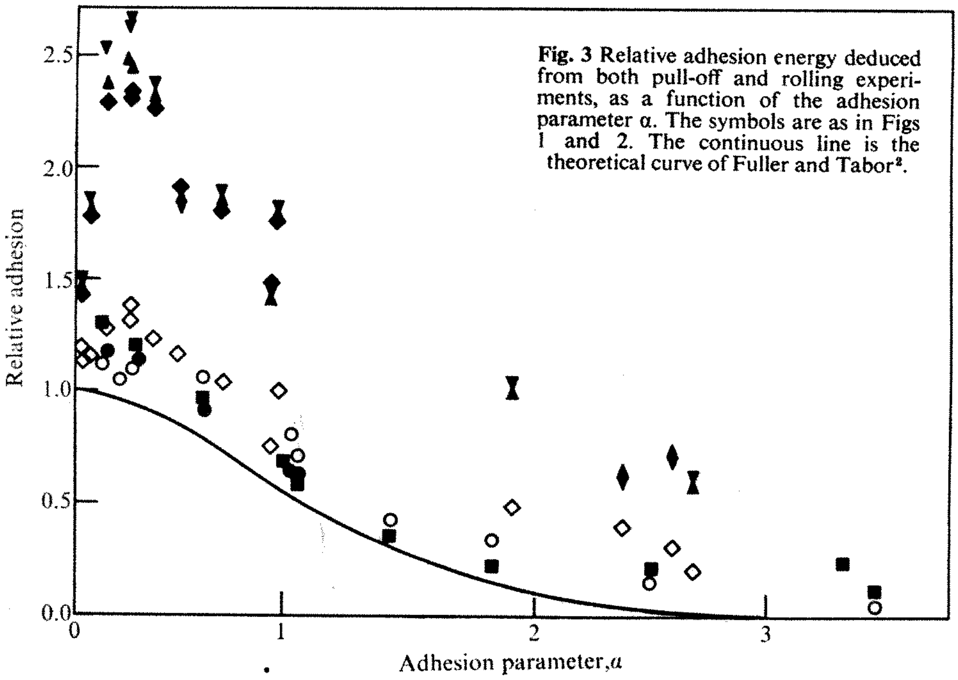

Fig. 3 Relative adhesion energy deduced from both pull-off and rolling experiments, as a function of the adhesion parameter α. The symbols are as in Figs 1 and 2. The continuous line is the theoretical curve of Fuller and Tabor[2].

greater than the adhesion with a nominally smooth surface. This effect is greatest when the effective surface energy is large and the modulus is small, and it is especially marked in the case of rolling on soft rubber. These results are shown in Fig. 3, where adhesion (normalised to the adhesion against a nominally smooth surface under the given conditions) is plotted against the adhesion parameter α. The theoretical curve of Fuller and Tabor is shown by the continuous line. The adhesion parameter seems to be a useful way of describing the influence of topography on the normalised adhesion, all the curves falling to half their maximum value when $\alpha \simeq 1$.

The increase of the adhesion for small roughnesses may be accounted for as follows: when a hard sphere is separated away from an elastic surface, there exists a minimum stable contact area. At this point the stored elastic energy is 1.4 times greater than the surface energy of the contact area. If the separation is increased quasi-statically, the rubber pulls away completely from the spherical surface, and all this elastic energy is lost. If the rate of separation is increased, then, because of viscoelastic effects, the elastic energy lost may be greater still.

For almost smooth surfaces, most of the contact areas between asperities and the rubber are smaller than the critical value and the surfaces therefore peel apart in a similar manner to perfectly smooth surfaces. For very larger roughnesses, the reduction in the true area of contact dominates. But between these two extremes a substantial proportion of the asperities may have a contact area just greater than the critical value, and therefore an increase in adhesion of up to 40% is possible. Simple analysis indicates that α should be an adequate parameter to determine the roughness at which this effect is important with the peak at $\alpha \simeq 0.2$–0.3. Comparison of these predictions with Fig. 3 is very satisfactory.

It will be noted that there is an exceptionally large increase in normalised adhesion in the case of the rolling experiments on very soft rubber. This may be due partly to the viscoelastic effect described above, and also to a snowballing of the adhesion (the extra adhesion at the rear of a rolling cylinder pushes more asperities into the rubber at the front, which increases the adhesion and so on).

A detailed account of the theory is in preparation. We wish to thank Professor D. Tabor for advice and encouragement and also Dr Whitehouse and Mr D. Kinsey of Rank Taylor Hobson for their help with the surface topography measurements. We are grateful to the SRC for an ASSIST award to G.A.D.B.

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Received February 2; accepted February 13, 1976.

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Pitting corrosion as a stochastic process

We believe that a stochastic theory originally developed to explain the fracture of solid materials under stress can be used to explain pitting corrosion. In the past (see ref. 1), both pitting potential[2] and induction time[3] have been used to select resistant alloys and to search for possible breakdown mechanisms. Studies of these and other phenomena, however, always reveal a wide scatter of data, so we have used the stochastic approach, which has proved useful in understanding time-dependent phenomena (see refs 4 and 5).

Pitting corrosion can be regarded as the failure of a two-dimensional film, which is altered over a finite time. Thus a stochastic approach is needed to explain many of the unsolved problems of pitting corrosion, such as the size effect and sweep rate sensitivity of pitting potential and the relationship between pitting potential and induction time.

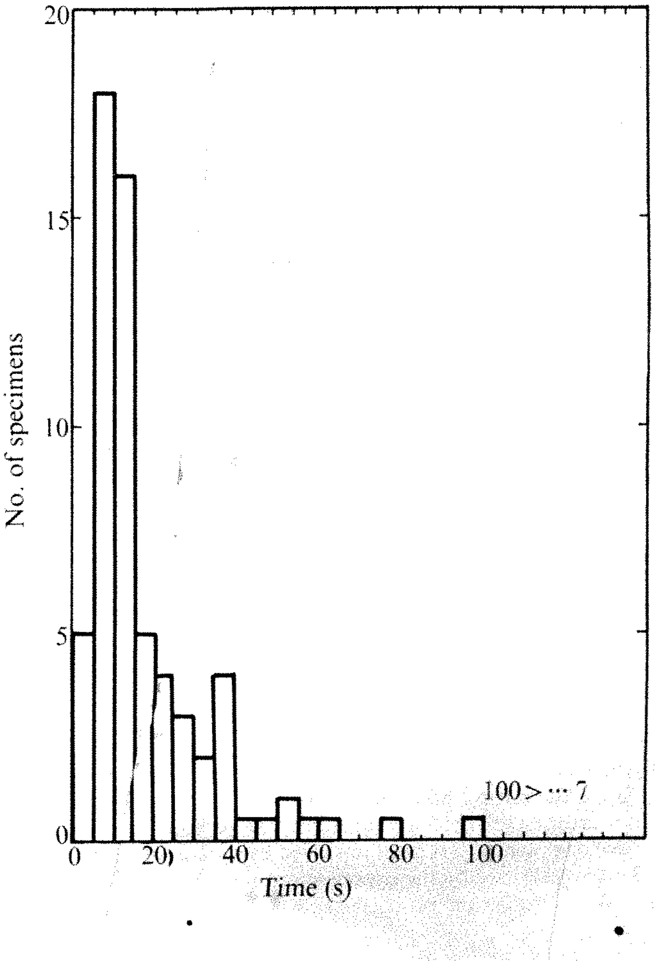
The stochastic approach requires the measurement of several series of data taken during the pitting process, in the same controlled conditions. We have developed a multichannel pitting corrosion tester which can measure the pitting potential or induction time of 12 specimens consecutively, using one potentiostat. Figure 1 is a histogram of induction time for 72 specimens of 18-8 stainless steel, the potential of which was kept constant at 0.35 V in 3.5% NaCl solution saturated with nitrogen gas at 35 °C. Though further experiments revealed that variations in pitting potential obey the normal distribution a distorted type of distribution was observed for the induction time.

This behaviour can be explained using the stochastic theory developed[4] for the brittle fracture of glass. The transition probability, $\lambda(t)$, of pit formation at any time, t, after applying the potential, is given by

$$\lambda(t)\mathrm{d}t = -\mathrm{d}P(t)/P(t) = -\mathrm{d}\ln P(t) \qquad (1)$$

where $P(t)$ is the survival probability, that is, the number of

Fig. 1 Histogram of induction time for pitting at constant potential (0.35 V) for 304 stainless steel in 3.5% NaCl solution saturated with nitrogen gas at 35 °C (72 specimens). Surface of specimens was polished with 6/0 emery paper before immersion into solution. After holding specimens in natural corrosion state for 5 min, and for further 5 min at −0.10 V to give a constant surface condition, the potential of the specimens was shifted to +0.35 V, at which potential pitting occurred after the induction time.



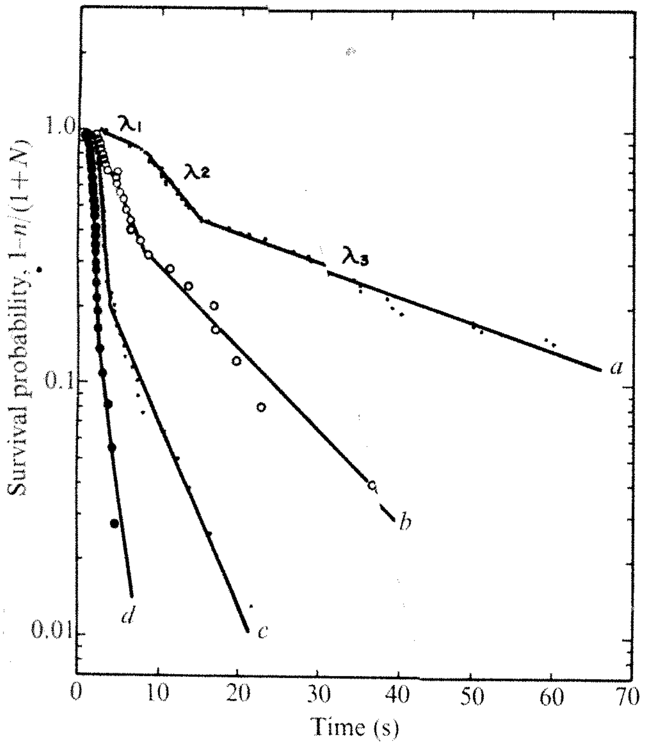


Fig. 2 Change in survival probability with time *a*, 0.35 V; *b*, 0.36 V; *c*, 0.37 V; *d*, 0.378 V. The slope of lg*P* against time gives transition probability

non-pitted specimens at time t. This equation is integrated for $P(0) = 1$, giving

$$P(t) = \exp(-\int_0^t \lambda(t)\mathrm{d}t) \tag{2}$$

Generally, $\lambda(t)$ is a function of time, but if it is not, equation (2) can be transformed to

$$\ln P(t) = -\lambda t \tag{3}$$

Equation (3) predicts a linear relationship between the logarithm of survival probability and the time elapsed after applying the potential. The survival probability is defined as $1 - n/(1+N)$, where N is the total number of specimens and n is the number of specimens failed in time t. Figure 2 shows such a plot for various potentials; the straight line which is divided into three. The slope of the lines gives the transition probability, as understood from equation (3).

The three distinct divisions could correspond to three processes causing pit formation at any one potential. The first process has the lower transition probability of λ_1, compared with λ_2 for the second process, with almost no delay. The second process begins only after a delay, in spite of the higher transition probability, λ_2. The third process has the lowest transition probability of λ_3, as shown in Fig. 2. All are highly dependent on potential, and λ_1 in the first process is described by

$$\begin{aligned}\lambda_1 &= \alpha_1(E - E_{\mathrm{crit}})\\ &= 8.20(E - 0.346)\end{aligned} \tag{4}$$

where E is the potential applied to the specimen in volts, and E_{crit} is the critical potential, at which no pitting is expected. It is interesting to note that the same linear dependence of induction time on potential has been observed by Sato *et al.*[6]. Both λ_2 and λ_3 are described by the exponential function of potential.

When transition probability is a function of potential, the most probable value of the pitting potential can be obtained using the equation[5]

$$\nu \mathrm{d}\lambda/\mathrm{d}E|_{E=\bar{E}} = \lambda^2 \tag{5}$$

which is derived by differentiation of the probability density function of pitting potential, where ν is the sweep rate of potential and $\bar{E}$ is the most probable value or mean value of pitting potential. If the first process determines the pitting potential, the sweep rate sensitivity of pitting potential can be obtained by the substitution of λ_1 into λ in equation (5)

$$\begin{aligned}\bar{E} &= (\nu/\alpha_1)^{\frac{1}{2}} + E_{\mathrm{crit}}\\ &= 0.349\nu^{\frac{1}{2}} + 0.346\end{aligned} \tag{6}$$

This predicted relationship has been confirmed in another set of experiments for determining E with changing potential sweep rates. This indicates that only the first process operates to cause pitting potential. It should also be emphasised that no paper has ever mentioned such dependence of pitting potential on the square root of the potential sweep rate. This relationship could be used to determine the value of the critical potential by extrapolating to the zero sweep rate even if the potential sweep method is used. The stochastic theory also predicts that the lowest pitting potential can be expected on the largest area of specimen. This could explain the unexpected failures which often occur in practical large scale apparatus in spite of proved resistances in laboratory tests using specimens with small areas.

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Received January 12; accepted January 21, 1976.

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Primary production of oxygen from irradiated water as an explanation for decreased radiobiological oxygen enhancement at high LET

WE report here the results of sensitive measurements of oxygen yields in water irradiated with radiation of high linear energy transfer (LET), in conditions which precluded the formation of oxygen in secondary reactions. These yields are important in radiobiology because, although small, they are sufficient to account for the decrease in value of the oxygen enhancement ratio found for biological cells irradiated by radiation of high LET compared with the value for low LET radiation.

An important feature of radiation damage to biological cells is that their sensitivity is greater in the presence of oxygen than under anoxic conditions[1,2]. For a given type of radiation the ratio of the two sensitivities, known as the oxygen enhancement ratio (OER) increases with increasing oxygen concentration. With radiation of increased LET, the anoxic sensitivity to radiation damage is increased, but the OER for a given oxygen concentration is decreased. Among the hypotheses advanced to explain these phenomena either independently, or as parts of a single mechanism, is the suggestion that the reason for decreased values of OER at high LET is the presence of oxygen

Table 1 G values of primary HO_2 and O_2

Ion or radiation	$^{60}Co\gamma$	^{4}He	^{4}He	^{14}N	^{14}N	^{14}N	^{14}N	^{14}N	^{20}Ne
Energy (MeV)	1.25	29	6	127	87	45	38	34	30
$G(HO_2)$	0.03	0.075	0.08	0.11	0.12	0.20	0.22	0.25	0.25
$G(O_2)$	0	0.0016	0.008	0.007	0.0085	0.012	0.012	–	0.031

formed in, or close to, the track of the ionising particle[3]. As a result, the radiobiological sensitivity is already raised above the hypothetical anoxic level, making further addition of oxygen less effective than with radiation of low LET, for which oxygen is not a radiolytic product. Alper and Bryant[2] have shown that the 'oxygen-in-the-track' hypothesis is superior to other hypotheses, for example, the suggestion that radicals formed in the track may recombine if sufficiently close together, thereby fixing damage without involving oxygen[4]. Experiments based on the oxygen-in-the-track hypothesis have been interpreted to quantify, by inference, an effective concentration of the oxygen present in cells along the track of energetic ^4He ions at the time of radiobiological action. Our measurements now provide direct radiation-chemical evidence that oxygen is formed as a primary product in sufficient yield to account for the decrease in OER at high LET.

Oxygen can be formed both with low and high LET radiation by the familiar bulk secondary reactions[5] of the normally accepted radiolytic products (OH, HO_2, H_2O_2, OH^-, H^+, H, H_2, e_{aq}^-) such as

$$OH + H_2O_2 \rightarrow HO_2 + H_2O \qquad (1)$$
$$OH + HO_2 \rightarrow O_2 + H_2O \qquad (2)$$
$$2HO_2 \rightarrow H_2O_2 + O_2 \qquad (3)$$

These secondary reactions are, however, suppressed by low concentrations of solutes, or are negligible in the time scale of spur and track reactions ($\leqslant 10^{-8}$ s) so that even reaction (1) cannot be invoked as an explanation for the formation of HO_2 in the track[6,7] because of its low rate constant.

Our method of preventing the occurrence of such secondary reactions and of measuring reproducibly the small primary yields of oxygen was as follows. Argon was bubbled through a suitable aqueous solution, irradiated by heavy ions from the Harwell Variable Energy Cyclotron at low currents (~ 2 nA) with adequate stirring, and then through a Hersch cell[8], the current from which gives a measure of the oxygen concentration in its own electrolyte. The area above the baseline of the graph of Hersch cell current against time obtained with constant argon flow provides a quantity proportional to the amount of oxygen formed during irradiation, which was converted to an absolute quantity by calibration with an electrolytic cell, also in the gas circuit. The methods of particle charge and energy measurement needed to obtain the yield per 100 eV, the G value, have been described[9]. By the use of methods and various solutes (unpublished work), measurements of oxygen yields have been used to obtain primary G values of HO_2, O_2, H_2O_2 and H_2 for γ rays, and some of these for several different kinds of radiation.

The principle of the method to measure HO_2 is to generate oxygen by the reaction of HO_2, with cupric ion[10,11]

$$HO_2 + Cu^{2+} \rightarrow Cu^+ + H^+ + O_2 \qquad (4)$$

in a deaerated solution of ferrous ammonium sulphate (10^{-3} M) and cupric sulphate (10^{-2} M) adjusted to $pH = 2$ with sulphuric acid. Other relevant reactions are

$$OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \qquad (5)$$
$$Cu^+ + Fe^{3+} \rightarrow Fe^{2+} + Cu^{2+} \qquad (6)$$
$$e_{aq}^- + H^+ \rightarrow H \qquad (7)$$
$$H + Cu^{2+} \rightarrow Cu^+ + H^+ \qquad (8)$$
$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH^- \qquad (9)$$

Any primary oxygen yield will also be measured from this solution and so must be subtracted from the overall oxygen yield.

The yield of O_2 was obtained as the lowest limiting yield when the concentration of Fe^{2+} ions was increased in deaerated Fricke solutions (ferrous ammonium sulphate 10^{-3} M, sulphuric acid 0.4 M), and was usually obtained between 10^{-2} and 10^{-1} M Fe^{2+}. The relevant overall reactions[12,13] are (5), (7), (9), (10) and (11)

$$H^+ + Fe^{2+} + HO_2 \rightarrow Fe^{3+} + H_2O_2 \qquad (10)$$
$$H^+ + H + Fe^{+2} \rightarrow Fe^{3+} + H_2 \qquad (11)$$

Some results are shown in Table 1.

The mean instantaneous concentration of a track product c_o mol l^{-1} can be derived from the initial track G value G_0, the mean LET, L (eV nm^{-1}) and the mean track radius r_o (nm): $c_o = G_0L/60\pi r_o^2$). In the absence of further reaction c_o will change with time to give c_t dependent on time

$$c_t = G_0L/60\pi(r_o^2 + 4Dt)$$

where t is the time (s) and D ($nm^2\,s^{-1}$) is the diffusion constant. For typical α-particle values[14] of L and r_o, 10^2 and 1 respectively, c_o for oxygen would be 4.2 mM and after 10^{-8} s, c_t would be 51 μM (taking $D = 2.1 \times 10^9$ $nm^2\,s^{-1}$ (see ref. 15)) if G_0 were assumed to be the measured value. If, however, oxygen were generated in the track core, the diffusion theory suggests[16] that much of it would be converted to O_2^- and HO_2 by track reactions (12) and (13)

$$e_{aq}^- + O_2 \rightarrow O_2^- \qquad (12)$$
$$H + O_2 \rightarrow HO_2 \qquad (13)$$

possibly giving rise to the yield of HO_2 measured in acid solution. Initial track core concentrations of oxygen may therefore be higher than c_o given above, and this is being investigated.

The way in which oxygen increases radiation sensitivity may be by reaction with lesions in the biological material formed by direct (or indirect) action in the δ-ray sheath surrounding the track core. For α particles of 5 MeV (90 eV nm^{-1}) Alper and Bryant find an OER for survival of 1.5 for two biological systems, *Chlamydomonas* and *Shigella*[2], for which the OER for γ-ray survival is 2.5. Using a mechanism based on competition between repair and fixation derived from data for low LET radiation they estimate an effective stationary oxygen concentration in the irradiated track volume of 2.2 μM. By interpolation of the results in Table 1 for 29 MeV and 6 MeV α particles (which are based on the average LET for the whole track) it can be shown that the G value for oxygen is ~ 0.005. The concentration of 2.2 μM together with this G value implies an irradiated track volume with a radius of 33 nm, in which it can be shown[17] ~ 90% of the dose is initially deposited. This indicates that the small yield of oxygen is sufficient to produce the observed effect.

The above argument has been simplified in that variations of oxygen concentration with time, and therefore of its reaction rate, have been ignored. More detailed calculations[16], however, including diffusion, using the same parameters for initial track geometry and the repair fixation competition confirm the adequacy of the oxygen yield. In addition, the reaction times are within those required[18] for the oxygen effect. Work in the determination of oxygen and HO_2 yields at varying Cu^{2+} and Fe^{2+} concentrations, and on oxygen enhancement ratios for radiation types with similar LET but differing secondary electron spectra, is in progress to test conclusions.

We wish to thank Messrs P. E. J. Abbott, W. R. Marsh, Dr R. May and Miss R. Cochrane for experimental assistance and advice and Drs C. B. Amphlett, A. J. Tench and J. Vennart for encouragement.

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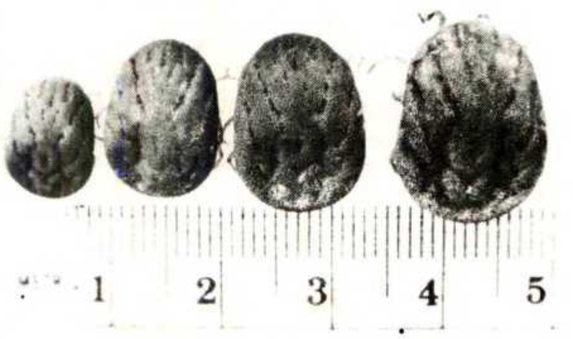
Ecdysone and the super tick

TICKS and insects develop and grow through intermittent replacement of their integument. This moulting does not occur beyond the adult stage, with the exception of primitive apterygote insects such as silverfish, which continue to moult and grow after they become sexually mature. Moulting ceases to occur in the pterygote adult insect because the glands secreting ecdysone, the hormone required to induce moulting, break down and disappear soon after the insect (and presumably also the tick) becomes adult[1]. The adult tissues of both hemimetabolous insects and those with complete metamorphosis continue to respond to ecdysone if exposed to it. There are, however, differences in the responsiveness of different segments of the insects and the resulting 'super-moulted' adult insects are usually defective[2–5]. We have found that ticks also retain the full potential to moult, grow and reproduce normally as adults if they receive ecdysone. Through successive adult moults 'super ticks' are obtained (Fig. 1) which consume very large volumes of blood and produce many more viable eggs than normal ticks.

Fig. 1 From the left, a normal fully engorged female compared with females super-moulted once, twice and three times.



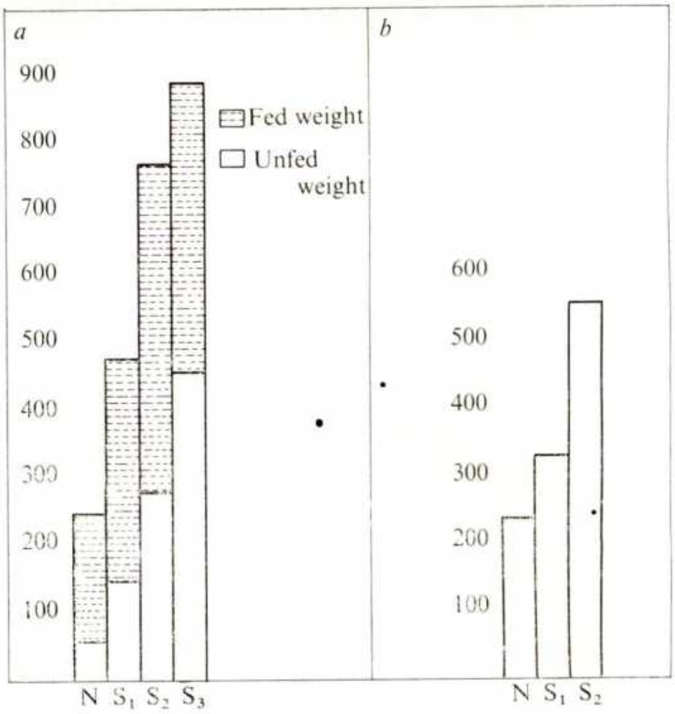


Fig. 2 The effects of ecdysone on body weight (*a*) and egg output (*b*) of female *Ornithodoros moubata* (w) super-moulted once (S_1), twice (S_2) and three times (S_3). Shaded areas, fed weight; unshaded areas, unfed weight (in mg).

We used the eyeless tampan, *Ornithodoros moubata* (Murray) (Argasidae), which is widely distributed throughout East Africa. We used adult ticks which were at least 1 month past their last moult. Females of this age are usually ready to take a blood meal and mate, and then lay about 250 eggs. Subsequent meals are necessary for additional batches of eggs. Ticks were divided into groups of 30 and fed on defibrinated pig blood through bat's membrane[6]. β-Ecdysone or the phytoecdysone ponasterone A were dissolved in 95% ethanol and added to the blood meal in concentrations of 1–5 μg ml^{-1}, the range which induces moulting in *O. moubata*[7]. After moulting, the ticks were offered a blood meal free of ecdysone, and then mated to assess their reproductive capacity. Another group was allowed to feed and moult as in the first group, and then fed again on blood containing ecdysone. The procedure was repeated again with the ticks that moulted on the second blood meal.

As Table 1 shows, if the blood contained ecdysone in a concentration greater than 2 μg ml^{-1}, most of the females moulted. With 4–5 μg ml^{-1} many of the moulted females underwent a second moult—without a further blood meal. Thus it seems that ingested ecdysone is not destroyed during digestion in ticks, and continues to stimulate moulting when the contents of the gut are being absorbed. This effect is dose dependent and seems to be rather rare at the lower concentrations.

Adult males seem more refractory than females to induction of moulting by ecdysone. Many of them, however, secreted new cuticle that was only loosely attached and could be peeled away easily. These males always died. Ponasterone A, which is more active than β-ecdysone when fed to insects[8], was more effective on ticks too, and at a dose of 5 μg ml^{-1} was highly toxic.

If females were mated and then fed on blood containing ecdysone, they moulted first, and if this process did not completely drain the food reserve, the remainder was utilised for egg production. Thus in one experiment 50 inseminated females were fed blood containing β-ecdysone

Table 1 Effect of β-ecdysone and ponasterone A on moulting of adult *Ornithodoros moubata*

Dosage (µg per ml blood)		No. super-moulted once	No. super-moulted twice*	No. of unsuccessful moults (in the first or second moult)	Overall death from treatment
β-ecdysone					
0	M	0	0	0	0
	F	0	0	0	0
1	M	4	0	9	10
	F	4	0	1	9
2	M	3	0	17	17
	F	21	2	3	3
3	M	3	0	15	15
	F	26	5	5	7
4	M	14	0	14	15
	F	29	15	4	4
5	M	17	0	11	20
	F	27	15	20	12
Ponasterone A					
0	M	0	0	0	0
	F	0	0	0	1
2	M	10	0	0	6
	F	19	0	0	1
3	M	4	0	10	10
	F	25	0	4	5
4	M	3	0	23	27
	F	19	0	8	10
5	M	8	0	13	20
	F	4	0	7	26

Each group contains 30 ticks.
*Moulting twice without a second blood meal.

(5 µg ml^{-1}). Twelve died and 38 moulted. Six out of the 38 then laid some eggs.

When super-moulted females were mated and fed blood, they consumed a much larger meal and consequently laid many more eggs than the normal tick. Blood utilisation seemed to be similar in normal and super-moulted ticks whether they moulted once or twice: for each 1 mg of blood consumed one egg was deposited (Fig. 2).

After one moult ticks were fed again on blood containing ecdysone (3 µg ml^{-1}); 57 out of 95 moulted after that meal. These twice-moulted ticks were about twice as heavy as the once-moulted adults, and about five times heavier than normal adult females. They consumed about 500 mg of blood and laid about 500 viable eggs. The larvae that hatched from these eggs developed normally. Only about four out of 12 females moulted after a third blood meal containing ecdysone. At this stage the weight of the unfed tick was nine times that of the normal tick (Fig. 2).

Our results may have some practical consequences as the use of ecdysone and its analogues has been proposed as a means of controlling ticks[10]. Obviously 'super ticks', produced by the application of ecdysone or its analogues, would constitute a danger of increasing the damage done by this pest rather than controlling it.

Ponasterone A belongs to a group of ecdysones widespread in large concentrations in plants. Many of them act in lower concentrations than does insect ecdysone[8], and it is conceivable that host animals might feed on plants containing large amounts of these substances, thus maintaining in their blood sufficient concentrations to induce super-moults in ticks. We have collected mature adult ticks from warthog burrows in the Nairobi National Park that were much bigger than ticks from the same source bred in the laboratory on pig blood. The possibility that warthogs feed on plants containing these phytoecdysones merits investigation.

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Received December 22, 1975; accepted January 30, 1976.

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Transitory hemizygosity of paternally derived alleles in hybrid trout embryos

IT is generally assumed that in species with yolk-rich eggs early embryonic development is sustained by maternally transmitted storage substances in the egg cytoplasm[1,2], while embryonic genes become activated later in development. As a rule, the maternally and paternally derived alleles of a particular gene locus start to function simultaneously at a defined developmental stage. But preferential activation of the maternally derived allele has been observed[3-7]. This prompted the suggestion[2,4] that the information required to switch on embryonic structural gene loci is transcribed by the diploid nucleus of the mother during oogenesis. According to this hypothesis, regulatory substances from the egg cytoplasm bind to receptor sequences adjacent to the structural genes, and thus induce their initial derepression. When the parentally derived receptor sequences are identical, the two alleles at the respective structural gene locus become activated synchronously. But when the base sequence of the paternally derived receptor site is different from the maternally transmitted one, for example when the two haploid genomes stem from different species, the recognition of the paternally derived receptor sequence by the regulatory substance of the egg might become difficult or even impossible. This would lead to either transitory or permanent exclusive expression of the maternally transmitted structural gene. Using the first appearance of embryonically specified α-glycerophosphate dehydrogenase (α-GPDH) isozymes as markers during the ontogeny of interspecific hybrid trout, we have now observed preferential activation of the paternally derived alleles. This seems to contradict the model of gene activation outlined here.

Brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) are easily hybridised to produce viable but sterile

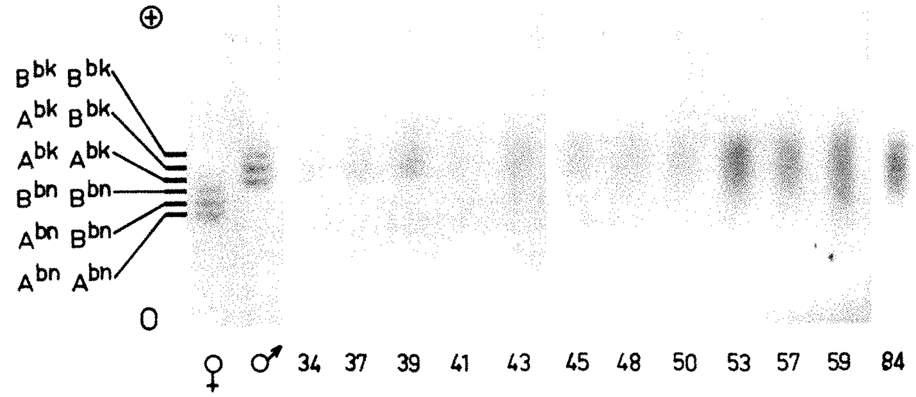

Fig. 1 Starch gel electrophoresis of α-GPDH isozymes from parental skeletal muscle tissue and single embryos at different developmental stages (age after fertilisation in days). Skeletal muscle (1:1 w/v) and embryos (about 1:0.25 w/v) were homogenised in 0.01 M phosphate buffer, *p*H 7.5, containing 10^{-4} M $MgCl_2$. The 20,000*g* (skeletal muscle) and 10,500*g* (embryos) supernatants were subjected to electrophoresis. Gels were made of 14% starch in 0.008 M Tris-0.003 M citrate buffer, *p*H 8.0; bridge: 0.223 M Tris-0.068 M citrate buffer, *p*H 8.0; run for 5 h at 12 V cm^{-1}. Gel slices were stained as described before[9].

hybrids. The time sequence of embryonic development in the offspring of intraspecific and interspecific matings is identical[8]. Both species have two ubiquitously expressed gene loci coding for a α-GPDH (EC 1.1.1.8), an enzyme of apparently dimeric structure[8,9]. The wild-type isozyme patterns in the two species consist of electrophoretically distinct triplets, of which the subunit designations are given in Fig. 1.

No α-GPDH staining activity was observed in unfertilised brown trout eggs. In the offspring of a mating between a female brown trout (phenotype $A^{bn}B^{bn}$) and a male brook trout (phenotype $A^{bk}B^{bk}$) staining activity was observed for the first time on day 34 after fertilisation (Witschi stage 20). The stain clearly was located in the position of the paternal type of isozymes, and the pattern remained unchanged until day 52. On day 53 (Witschi stage 23) enzyme activity in the position of the maternal species was visible for the first time. On day 84 (shortly before the resorption of the yolk sac) all of a sample of ten embryos showed the expected $A^{bn}B^{bn}$ $A^{bk}B^{bk}$ phenotype, exhibiting an electrophoretic pattern of seven bands (Fig. 1). Our study was extended also to the offspring of the reciprocal and the intraspecific matings. In these cases the maternally and paternally derived α-GPDH genes seemed to become activated simultaneously. (These findings will be reported elsewhere in detail.)

The preferential activation of paternally derived α-GPDH alleles seems incompatible with the model of gene activation in embryonic development outlined above. If, however, one assumes that a genetic polymorphism exists at a common receptor site adjacent to both of the two α-GPDH structural loci (preliminary results indicate close linkage of the two loci[8]) our finding becomes reconcilable with the model. The brown trout mother should have passed on to all of her offspring a variant receptor sequence, characterised by a lowered affinity to the regulatory substance compared with the paternally transmitted receptor sequence. Only when the cellular concentration of the regulatory substance has reached a threshold, possibly also by synthetic activity of the embryonic genome, can the maternally derived α-GPDH loci be switched on.

It is conceivable that mutational events could alter receptor sequences so that they will never be recognised by the regulatory substances. In fact, permanent repression of the maternal allele was observed in interspecific *Drosophila*[10] and sunfish[11,12] hybrids. Whitt *et al.*[12] suggested that permanent maternal allele inhibition is due to a repressor molecule produced by the paternal genome which, when associated with the paternal DNA, can react appropriately to specific gene-activating stimuli, but when associated with the maternal DNA, with a different nucleotide sequence, cannot respond. This model, based on observations with adult animals is obviously difficult to apply to early development, whereas genetic variability at receptor sequences might explain either set of findings.

If receptor sequences can occur in different allelic forms, preferential activation of paternally inherited genes should also occur in the offspring of intraspecific matings. We are testing this hypothesis. We thank Fischzuchtmeister H. Scheiffelen, Alpirsbach, Black Forest, for breeding the hybrids.

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Received December 15, 1975; accepted January 13, 1976.

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Increased frequency of heterozygotes for α_1 antitrypsin variants in individuals with either sex chromosome mosaicism or trisomy 21

THERE have been two reports[1,2] of an association of alpha-1 antitrypsin (α_1AT) variants and sex chromosome mosaicism. The data suggest that decreased α_1AT activity, as found in individuals heterozygous or homozygous for α_1AT variants, gives rise to abnormal chromosome segregation during mitosis. We report here further data which support those previous studies and suggest that decreased α_1AT activity is also an aetiological factor in trisomy 21.

α_1AT is produced by the liver and found in blood, semen, cervical mucus and other body fluids. It is a readily detectable, polymorphic, codominantly inherited inhibitor of cellular proteases. The structural gene for α_1AT is not on chromosome 21; however, its exact location is not known at present[3].

Using acid starch gel electrophoresis[4] and immunofixation electrophoresis at *p*H 8.6 (ref. 5), we found that three of nineteen individuals with mosaic sex chromosome abnormalities had a heterozygous α_1AT phenotype (Table 1). Of the three, two had the M_{LAMB} variant of α_1AT. M_{LAMB} is a newly discovered variant with approximately 20% less activity than the usual M form of α_1AT and, in one survey, was found in less than 1% of individuals typed[5]. Given an average frequency of heterozygotes for all α_1AT variants of approximately 10% (ref. 6), the observed number of α_1AT heterozygotes (12) in the three studies of individuals with mosaic sex chromosome abnormalities ($N = 47$) is significantly greater than expected: $\chi^2 = 12.6$, $P = <0.001$. (For this and subsequent calculations we have assumed the expected population frequency of heterozygotes for α_1AT to be exactly 10%.)

Table 1 α_1AT phenotypes in patients with sex chromosome mosaicism

	α_1AT phenotype		
	MM	MM_{LAMB}	MZ
45,X/46,XX	1	1	
45,X/46,X,i(Xq)	4		1
45,X/46,X,i(Xq)/47,X,i(Xq),i(Xq)	1		
45,X/46,X,r(X)	2	1	
45,X/46,XY	4		
45,X/46,X,t(22qYp)*	1		
46,XY/47,XXY	1		
46,XY/47,XYY	2		

*Short arm of Y translocated to long arm of 22.

We have also performed α_1AT phenotyping on 60 institutionalised patients with karotype proven trisomy 21. Since advanced maternal age markedly affects the incidence of Down's syndrome, we collected samples from two groups of patients with trisomy 21. Group 1 consisted of 29 patients whose mothers were under 30 yr old at the time of the patient's birth (mean maternal age± s.e. = 25.0±0.51 yr). Group 2 consisted of 31 patients whose mothers were older than 35 yr at the time of the patient's birth (mean maternal age± s.e. = 40.0±0.38 yr). The mean ages of the patients in the two groups (29.7±1.9 and 26.4±1.8 yr, respectively) were not significantly different. α_1AT phenotyping was performed blind with respect to maternal age, and the age and sex of the patient. We found two α_1AT heterozygotes in group 1 and 14 in group 2 (Table 2). The frequency of α_1AT heterozygotes in the total of both groups is significantly greater than that of the general population ($P = <0.0001$). But the two groups are significantly different from each other ($P = <0.005$). Group 1 alone, compared with the general population, had no greater frequency of α_1AT heterozygotes; group 2 had a markedly greater frequency of α_1AT heterozygotes ($P = <0.0001$). Thus the significant association of trisomy 21 with variant α_1AT alleles seems to be confined to individuals born to mothers over 35.

These observations, if supported by more extensive studies, raise additional questions. How do advanced parental age and diminished α_1AT activity (associated with heterozygous variants of α_1AT) interact to produce chromosomally abnormal offspring? Does diminished α_1AT activity predispose to other types of chromosome aberrations? What role, if any, does α_1AT have in normal meiosis and mitosis? Answers to these questions will provide important insights into mechanisms which control chromosome segregation. One important practical benefit may be the identification by α_1AT phenotyping of individuals with a significantly increased risk of producing children with chromosome abnormalities.

Table 2 α_1AT phenotypes in patients with trisomy 21

Maternal age at the time of the patient's birth	α_1AT phenotype	No. of patients	Frequency of heterozygotes
Group 1 less than 30 yr old	MS	1	0.069
	MM_{LAMB}	1	
	MM	27	
	Total	29	
Group 2 more than 35 yr old	MS	7	0 452
	$M_{LAMB}S$	1	
	MZ	2	
	$M_{LAMB}Z$	1	
	MM_{LAMB}	3	
	MM	17	
	Total	31	

This work was supported in part by grants from the National Institutes of Health and the National Cystic Fibrosis Research Foundation

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Received December 4, 1975. accepted February 5, 1976.

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Cytoplasmic factor responsible for germinal vesicle breakdown and meiotic maturation in starfish oocyte

IN starfish, the first maturation division of oocytes within an ovary is arrested at a late stage of prophase. It is resumed after stimulation by 1-methyladenine (1-MA) produced in the follicle cells under the influence of the gonad-stimulating hormonal peptide, released from the nervous tissues[1–3]. 1-MA induces breakdown of the germinal vesicle and maturation of oocytes isolated in seawater[2]. When it causes maturation, 1-MA first acts on the oocyte surface[4,5] to bring about cytoplasmic maturation, which is manifest in the acquisition of fertilisability[6]. The change in the cortical region of the oocyte under the influence of 1-MA is believed to produce a cytoplasmic factor which induces germinal vesicle breakdown (GVBD), leading to the completion of oocyte maturation. We now report microinjection experiments that provide evidence of such a cytoplasmic factor in the starfish *Asterina pectinifera*.

An earlier experiment had shown that 1-MA injected directly into an immature oocyte failed to induce germinal vesicle breakdown even in a concentration higher than that which is effective when applied externally[4]. We found, however, that cytoplasm from an oocyte which is maturing after treatment with 1-MA can induce GVBD when injected into an immature oocyte by Hiramoto's method[7] (Fig 1) The frequency with which GVBD was induced in recipient oocytes was proportional to the volume of cytoplasm injected. Almost 100% breakdown was observed when the volume injected was 220 pl (about 1/15–1/20 of the oocyte volume). Germinal vesicle was broken down 20–40 min after microinjection, and so was follicular envelope, but GVBD was never observed when the cytoplasm of immature oocytes was injected as a control.

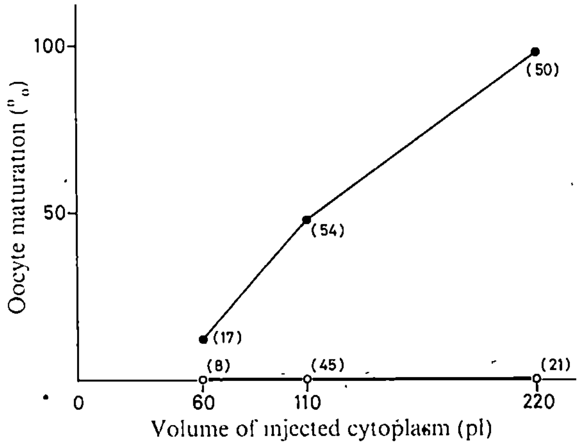
Cytological examination of oocytes which underwent

GVBD after microinjection of cytoplasm from 1-MA-treated oocytes showed that meiosis was completed normally. Recipient oocytes were fixed with ethanol–acetic acid (1 : 1) and stained with aceto-orcein. Each oocyte was crushed under a cover glass and the chromosomes were observed microscopically. Chromosome condensation was observed 35 min, metaphase about 45 min and anaphase about 55 min after injection. The first and second polar bodies were discharged 70 min and 100 min after injection, respectively. The egg pronucleus was observed 120 min after injection. The time course of meiosis of injected oocytes was the same as that of oocytes treated with 1-MA in 80% artificial seawater. Thus the maturing oocyte produces a cytoplasmic factor which induces GVBD, leading to the completion of meiotic maturation. Furthermore, on insemination, oocytes induced to mature by injection of maturing cytoplasm formed somewhat incomplete fertilisation membranes. They then cleaved and developed normally.

Next we investigated the changes in the maturation-inducing capacity of the cytoplasm of 1-MA-treated oocytes as they matured. Cytoplasm (about 220 pl) of 1-MA-treated oocytes, taken at 20-min intervals after treatment, was injected into immature oocytes with germinal vesicles. When oocytes were taken from the same ovary and treated with 1-MA in the same way as donor oocytes, the germinal vesicle broke down after 15–30 min, the first polar body was discharged after 60–70 min and the second polar body was discharged after 110–120 min. Injected cytoplasm was most effective in inducing GVBD 20–40 min after administration of 1-MA, and then its efficacy declined (Fig. 2). After 80 min, the cytoplasm had almost lost the capacity to induce germinal vesicle breakdown when injected into immature oocytes, although recipient oocytes matured normally when subsequently transferred to seawater containing 10^{-6} M 1-MA. Furthermore, maturation-inducing capacity was found in the cytoplasm of some oocytes 13 min after administration of 1-MA, when germinal vesicles remained intact. The activity thus does not seem to be the result of GVBD.

Cytoplasm of an oocyte undergoing maturation as a result of microinjection of maturing cytoplasm was injected successively into immature oocytes. In three replicates about 220 pl of cytoplasm taken just after GVBD, was injected into an oocyte with intact germinal vesicle. Since this volume of injected cytoplasm, 1/4 of which barely induced maturation (Fig 1), was about 1/15 of the oocyte volume, the cytoplasm of the original oocyte which matured in response to 10^{-5} M 1-MA was diluted 15^4 times after the fourth injection. In spite of such extensive dilution, the rates of maturation of the recipient oocyte were, respectively, 100% (18 of 18), 88% (15 of 17), 93% (14 of 15) and 92% (12 of 13) in the first, second, third and fourth injections. Thus the cytoplasmic factor able to induce maturation was amplified in recipient oocytes during maturation.

Fig. 1 GVBD induced by microinjection of cytoplasm taken from 1-MA-treated oocytes of *A. pectinifera* Donor oocytes were treated with artificial seawater (ASW) containing 10^{-5} M 1-MA for 10 min and washed with ASW. Cytoplasm was removed from donor oocytes 20–25 min after the commencement of treatment with 1-MA and injected into immature oocytes with intact germinal vesicle, which were then placed in 80% ASW. This concentration was more suitable than normal ASW for injecting a large quantity of cytoplasm ●, Cytoplasm from 1-MA-treated oocyte; ○, cytoplasm from intact immature oocyte. Number of injected oocytes is shown in parentheses.



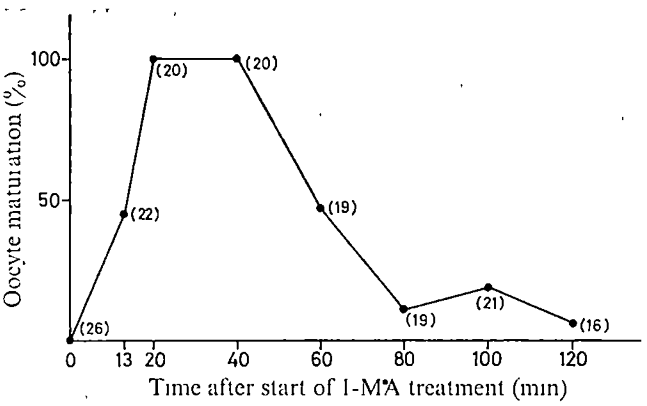


Fig. 2 Changes in the maturation-inducing capacity of the cytoplasm of 1-MA-treated oocytes during maturation in *A. pectinifera*. Donor oocytes were treated with ASW containing 10^{-5} M 1-MA for 10 min and washed with ASW. Number of injected oocytes is shown in parentheses.

These results are interesting when compared with data for oocyte maturation in frogs[8,9]. In *Rana pipiens*, pituitary gonadotropins seem to act on the follicular tissue to produce progesterone or a related substance which brings about oocyte maturation. Injection of progesterone into the oocyte fails to induce maturation, whereas external application to oocytes free of follicle cells is effective. But the cytoplasm of progesterone-treated frog oocytes can also induce maturation when injected into immature oocytes. Therefore, progesterone is considered to act externally on the oocyte surface to produce a cytoplasmic factor called "maturation-promoting factor", which causes the oocytes of frogs to mature In the starfish, gonad-stimulating hormonal peptide is secreted from the nervous system to produce 1-MA in the follicle cells. 1-MA acts on the oocyte surface to produce and release a new factor in the cytoplasm which is the true inducer of germinal vesicle breakdown and meiotic maturation. Further work should be directed towards the characterisation of this factor.

We thank Professor Y. Hiramoto for advice on microinjection and Professor J. C. Dan for reading the manuscript. This investigation was supported in part by grants-in-aid from the Japanese Ministry of Education and the Ford Foundation.

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Association of lipid oxidation with seed ageing and death

SEEDS usually exhibit their maximum germination potential soon after harvest, and as storage time increases, they lose vigour and eventually die. The rate of physiological ageing generally increases with increased moisture content and temperature[1]. In addition, certain xerophytic storage fungi (primarily *Aspergillus* spp.) can attack seeds in storage when seed moisture content is in equilibrium with relative humidities greater than 65% (ref. 1), and cause changes that mimic physiological ageing Physiological changes accompanying physiological ageing[1–5] and attack by storage fungi[5] have been catalogued, but the basic biochemical events leading to these changes are not known. Harrington[1] and Pammenter *et al.*[6], however, have suggested that oxidation of unsaturated lipids may lead to free-radical formation. Free radicals may then damage cellular membranes and react destructively with macromolecules. Similar suggestions have been presented to explain ageing in several other systems[7], and the results of experiments reported here indicate that free-radical formation may be important in seed deterioration. Theories of mechanisms of lipid oxidation indicate that fatty acids with two or more unsaturated bonds should be more labile and prone to form free radicals than more highly saturated acids[8,9]. Thus, if fatty acid oxidation and free-radical formation are occurring, the highly unsaturated acids should decrease as seeds deteriorate, whereas the quantity of saturated fats should remain constant in the absence of beta oxidation

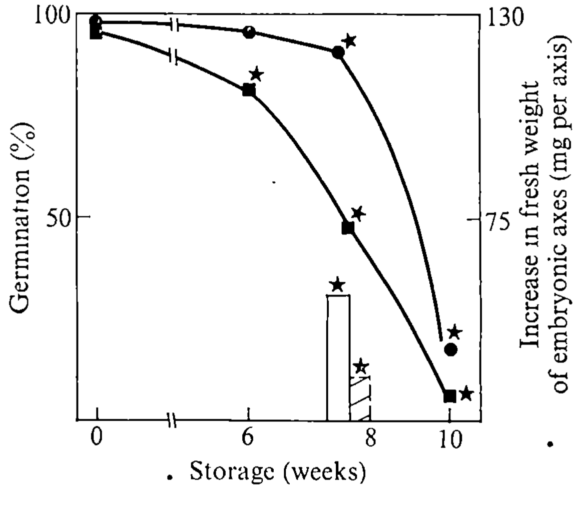
To determine whether these predicted changes occurred as a result of ageing and/or of infection by storage fungi, we used pea (*Pisum sativum* L. 'Alaska') seeds rendered microorganism-free or inoculated with *Aspergillus ruber* (Konig, Spieckermann, and Bremer) Thom and Church prepared as described elsewhere[5]. They were stored at 30 °C and 92% relative humidity. Periodically, seeds of different ages were removed from storage, and the percentage germination, the vigour, and the quantities of the major fatty acids in whole seeds and embryonic axes were determined. Seeds inoculated with *A ruber* were sampled only at 8 weeks, since visible infection (sporulation from the hila) does not commence until at least 6 weeks after inoculation. Only seeds with visible sporulation were used in these experiments. The percentage germination was determined by placing 30 seeds between sterile, wet paper towels, and counting the number of seeds germinating normally after 5 d incubation at 25 °C. A vigour rating was obtained by placing other seeds in the same conditions as for germination testing, and measuring the increase in fresh weight of the shoot–root axes after 3 d growth. This method measures the speed of germination and is correlated with several biochemical tests for vigour[5].

To determine the quantities of fatty acids, seeds were first lyophilised, and then ground to a fine powder in a Waring Blendor. Hydrolysis, methylation of fatty acids and extraction of the resulting esters was accomplished by a modification of the method of van Wijngaarden[10]. An aliquot (400 mg) of each subsample of ground pea seeds was placed in a 50-ml round bottomed flask, and 3 ml of 0.66 N methanolic KOH and 1 ml of heptadecanoic acid in methanol (1 mg ml^{-1} as an internal standard) were added to the flask The flask was then connected to a condenser, the mixture was brought to a slow boil for 5 min, 4 ml of BF_3 in methanol (14% w/v) was added through the condenser, and the mixture was boiled for a further 2 min Heptane was then added (1 ml), again through the condenser, the mixture boiled for an additional minute, and the condenser and flask then cooled rapidly in an ice bath. Sufficient saturated NaCl solution was added to the cooled mixture to bring it to the neck of the flask, and an aliquot of the heptane layer transferred to a vial containing a small amount of anhydrous Na_2SO_4. This heptane solution contained the fatty acid esters and was injected directly into the gas chromatograph. Separation was accomplished on a column (32 mm outside diameter and 1.8 m long) of Chromosorb WHP 100–120 mesh coated with 5% diethylene glycol succinate maintained at 150 °C. The carrier gas was N_2 at 10 ml min^{-1}. Detection was by a flame ionisation detector and quantitation was by integration of the area of each peak, corrected for the sensitivity of the detector relative to a heptadecanoic acid standard (determined earlier using known quantities of each of the fatty acid esters). The identities of the acid esters were verified by chromatography on 1% SE-30 (methyl), a non-polar gum rubber, using the same conditions as for the diethylene glycol succinate column. All experiments were conducted in triplicate.

The percentage germination of microorganism-free pea seeds decreased gradually from 0 to 8 weeks of storage, and then decreased markedly between 8 and 10 weeks (Fig. 1). Vigour, however, had decreased noticeably by 6 weeks of storage and declined more rapidly than germination during 8 weeks of storage. Between 8 and 10 weeks, vigour, like germination, decreased rapidly. The rapid decline of vigour compared with viability (germination) as a consequence of ageing has long been noted[12], but its cause is unknown. Seeds infected with *A. ruber* showed reduced vigour and germination compared with similar seeds stored in aseptic conditions.

The principal fatty acids of pea seeds were identified as palmitic, stearic, oleic, linoleic and linolenic acids. No treatment changed the concentrations of the saturated or monoenoic acids (palmitic, stearic, and oleic) in whole seeds or embryonic axes. The concentrations of these acids in whole seeds were 2.0, 0.50, and 5.1 mg per g dry weight, and those in axes were 1.8, 0.25, and 5.8 mg per g dry weight, respectively. The concentration of both the di- and trienoic acids (linoleic and linolenic), however, were affected by both storage and infection (Fig. 2). In whole seeds, the concentration of linoleic acid declined gradually with increased storage, and there was no additional decrease after infection. There was no significant change in linoleic acid concentration in the axes, larger changes were observed with linolenic acid: in whole seeds, only about 50% of linolenic acid remained after storage for 10 weeks with the most dramatic decrease of this acid occurring between

Fig. 1 Percentage germination of microorganism-free (●) and *Aspergillus ruber*-infected seeds (open column), and the increase in fresh weight of embryonic axes from microorganism-free (■) and *A. ruber*-infected seeds (hatched column) after 3 d germination Starred points (★) are significantly different (*K* = 100) from values obtained from unaged seeds according to the test of Waller and Duncan[14].



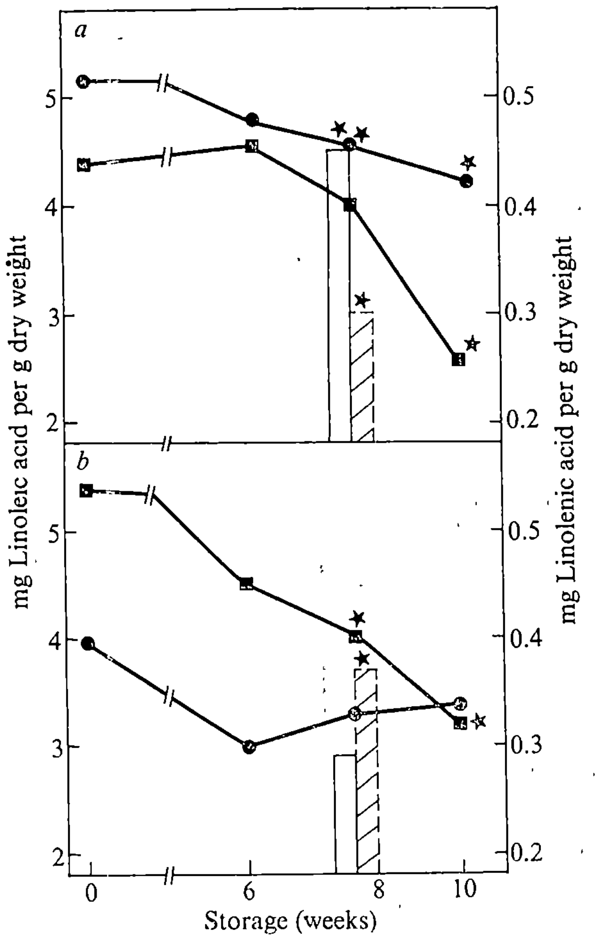


Fig. 2 Changes in the concentration of linoleic and linolenic acids in whole pea seeds (*a*) and in embryonic axes (*b*) after storage for various times The concentration of linoleic acid in microorganism-free (●) and in *Aspergillus ruber*-infected (open column), and the concentrations of linolenic acid in microorganism-free (■) and *A. ruber*-infected (hatched column) seeds are shown. Starred points (★) are significantly different ($K = 100$) from the levels obtained for unaged seeds according to the test of Waller and Duncan[14].

weeks 8 and 10. In embryonic axes, there was a rapid decrease in the quantity of linolenic acid with ageing, but no additional decrease as a result of infection by *A. ruber.*

These results suggest strongly that beta oxidation or other utilisation of fatty acids does not occur in stored seeds, since the saturated and monoenoic acids did not change in concentration. The rapid decrease in linolenic acid with ageing in axes roughly paralleled the loss of vigour of the seeds and suggests that free-radical formation may be a factor in loss of seed vigour and, subsequently, seed death. Changes occurring in the axes are probably of more consequence to the overall health of the seed than those occurring in the whole seeds (cotyledons, axes and seed coat), since only the axes are capable of growth. They should also be more sensitive to oxidation processes since they are rather small structures, comprising ~ 1% of the total seed weight, attached to the outer surface of the embryo Toxic materials formed in the cotyledons as a consequence of oxidation could, however, be transported to the axes and cause damage.

Changes in linoleic acid were less pronounced than changes in linolenic acid; this was expected since linoleic acid is less reactive than linolenic acid[9], and since it is present in an approximately tenfold higher concentration than linolenic, relatively small changes (less than ~ 0 5 mg g^{-1}) are difficult to detect.

A. ruber infection caused a statistically significant reduction in the quantity of linolenic acid in whole seeds, but not in axes (Fig. 2). Any explanation of this phenomenon must consider that *A. ruber* damages pea embryos by way of a diffusable substance, since the fungus is restricted to the seed coats and rarely invades living embryos[13]. The *A. ruber* toxin could somehow initiate oxidation without a pronounced effect on the subsequent oxidation rate. Thus, in axes where oxidation due to ageing occurred before infection was heavy, lipid oxidation would not be strongly affected by *A. ruber* infection, whereas in whole seeds (primarily cotyledons), the processes of initiation could still be occurring. More information on the mechanisms of oxidation as a consequence of ageing and infection is needed before any conclusions can be drawn on the relative effects of ageing or infection on the oxidation of fatty acids.

The correlations shown here, particularly between the disappearance of linolenic acid, loss of seed vigour and seed death, thus indicate that free radical formation may well be an important factor in seed deterioration

We thank Glenda Nash for technical assistance and John Barnard for statistical analyses.

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Received December 12, 1975, accepted January 23, 1976.

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Enrichment of antigen-specific helper and suppressor T cells

LYMPHOCYTES are known to differ on the basis of their antigen specificity, as postulated by Burnet[1], and experimentally confirmed by a variety of techniques[2–5]. Although there are many problems involved in purifying cells reactive to a given antigen there have been successful reports. These include the purification of rosette-forming cells[6], the separation of cells binding fluoresceinated antigen on a cell sorter[7], and the recovery of cells which have bound to an antigen matrix[8] or a cell surface[8–10]. The use of gelatin[8,11] substrates has proved to be successful in the purification of B lymphocytes, with enrichment of antigen-specific cells estimated in the order of 100-fold[11,12] Because antigen-binding T cells bind better at 37 °C than at 4 °C (refs 13 and 15), collagen gels were used in this study, as gelatin melts at 37 °C. A procedure for the enrichment of antigen-specific T cells should provide an essential step in the approach to many problems of understanding T cells and their function, such as the nature of T-cell receptors on subpopulations of T cells, the nature of mediators of T-cell function (especially antigen-specific mediators) and the mechanisms and control of T-cell activation. Here we describe a method for the selective enrichment of T cells reactive to protein or synthetic polypeptide antigens, and demonstrate the selectivity and specificity of the procedure by cross absorption experiments. The general applicability of the procedure was verified by using T cells immunised *in vivo* or *in vitro*, and by demonstrating 100-fold enrichment with different subpopulations of T cells, namely carrier-specific helper and carrier-specific suppressor cells involved in the *in vitro* antibody response.

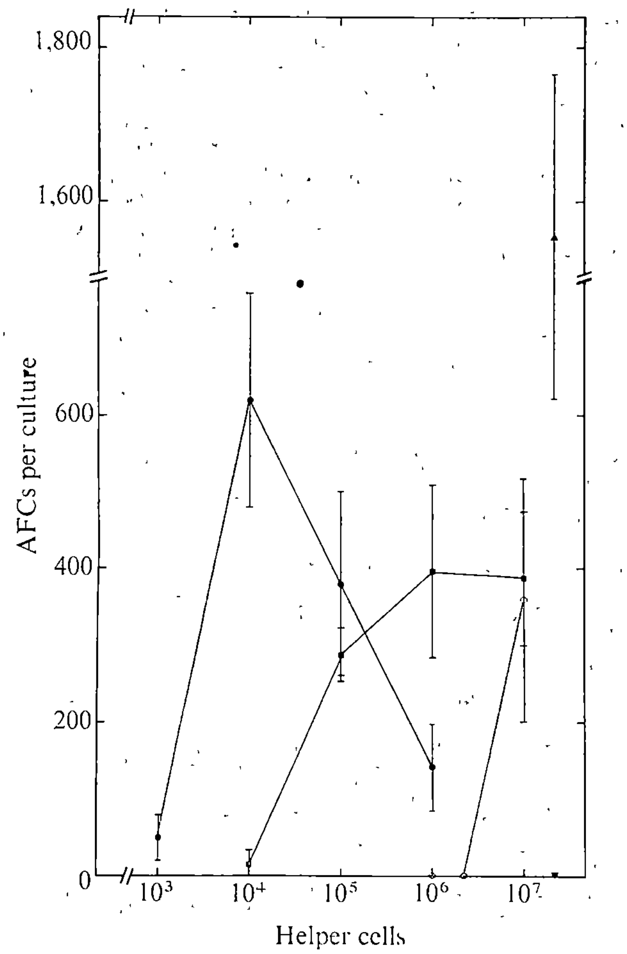


Fig. 1 Enrichment of helper cells from mice primed *in vivo*. ●, Adherent cells; ■, unfractionated cells; ○, non-adherent cells. Spleen T cells from mice primed 3 times with TNP–KLH adsorbed on bentonite[27] were passed through nylon wool[28]. The passed cells contained 4% cells stainable with fluoresceinated anti-Ig (B cells) and 95% cells stainable with anti θ. These cells were plated on keyhole limpet haemocyanin (KLH) derivatised collagen gels. Cell suspensions (20×10^6–35×10^6 cells per 2 ml) in HEPES (15 mM) buffered Eagle's (HEM) were incubated on a rocker for 60 min at 37 °C Non-adherent cells were removed by aspiration followed by 2 washes with HEM. Adherent cells were recovered from the gels by digestion with collagenase (Worthington Biochemicals Co), 180 μg per plate in 2 0 ml HEM for 90 min at 37 °C, followed by centrifugation and resuspension in a small volume of HEM containing 10% foetal calf serum. The specific binding of the populations used was 1–2% of the cells applied, and nonspecific binding, to collagen itself, or derivatised collagen (with another protein) was 0.5% The helper capacity of the 2 fractions was compared with that of the unfractionated T cell *in vitro*, using unprimed CBA spleen (sometimes anti-T treated spleen) as a source of B cells and 0.1 μg ml^{-1} trinitrophenylated KLH (TNP–KLH) as antigen. Cultures were performed in Marbrook–Diener flasks as described in detail elsewhere[25,29]. Antibody-forming cells to DNP or TNP were assayed after 4 d in culture[25,29] Gels used in this study were made from acid-soluble rat tail tendon collagen, 0.1% in 0.1 M acetic acid, layered in Petri dishes (usually Sterilin 50 mm), and the gels formed by exposure to ammonia Binding of antigen to the gels was performed following periodate oxidation of the gels with 0 1% sodium periodate (BDH) for 1 h at room temperature in the dark The gels were washed with phosphate-buffered saline (PBS) and the protein solution (usually 0 1–1.0 mg ml^{-1}) was added and left overnight The gels were then washed with PBS and HEM, and usually used immediately Occasionally they were stored in the cold for up to 4–5 d. Arithmetic mean ±s e m. of 3 cultures per group (for all groups). Analogous results were obtained in 7 experiments of this type with KLH and 5 with chicken gamma globulin.

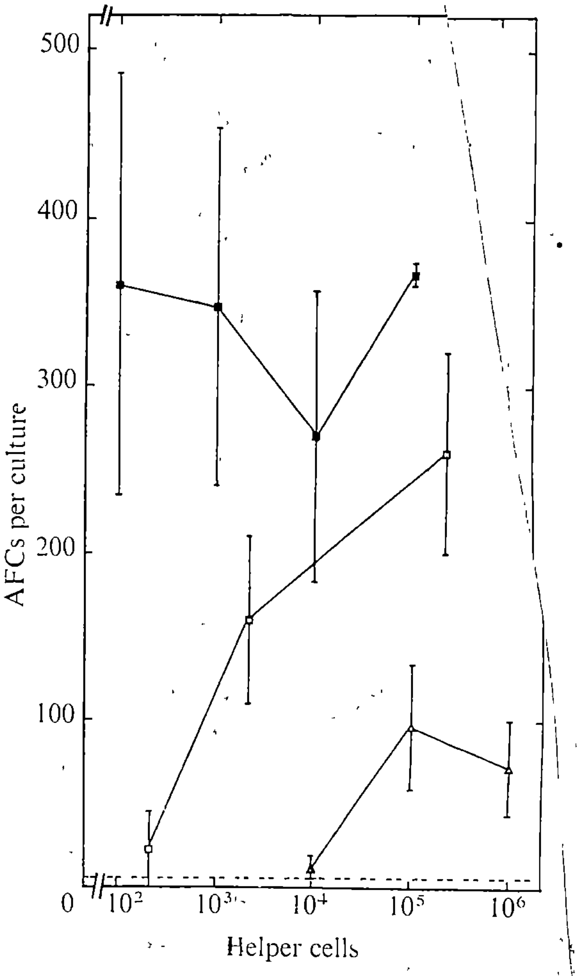


Fig. 2 Enrichment of *in vitro* primed helper cells to KLH ■, Adherent cells; □, unfractionated cells; △, non-adherent cells T-helper cells were induced by culture for 4 d in Marbrook flasks with low concentrations of antigen[16]. Surviving cells were plated on KLH gels and the adherent and non-adherent fractions were collected and compared for their helper capacity *in vitro*, as described in the legend to Fig. 1. As few as 100 adherent cells sufficed for an optimal helper response, compared with 10^5 of the unfractionated cells. 10^6 non-adherent cells were less efficient than 10^2 adherent, verifying that the technique both enriches and depletes for antigen-specific cells. Note that the depletion in this (and all other) experiment is not complete. Three experiments of this type have been performed with analogous results.

Nylon wool-purified T cells from mice primed to keyhole limpet haemocyanin (KLH) were incubated with KLH derivatised collagen (1 mg per 50-mm dish was found to be optimal) for 60 min on a rocker at 37 °C. The non-adherent cells were removed and the gels rinsed and digested with collagenase. In all experiments the viability of the adherent cells was about 90%.

The helper capacity of three fractions were compared: (1) the nylon-purified T cells, (2) the cells adherent to the KLH and (3) the non-adherent cells. Titrations were performed *in vitro*, comparing the numbers of *in vivo* primed T cells needed to generate anti-dinitrophenyl (anti-DNP) antibody responses, using normal spleen as a source of antibody-forming cell (AFC) precursors and triphenylated KLH (TNP–KLH) as antigen. As shown in Fig. 1, the adherent cells were about 30 times more efficient helper cells than the unfractionated T cells. The T cells which did not adhere to the KLH gels were depleted of helper cells by about 100-fold

Analogous experiments were also performed with helper cells obtained from tissue culture[16]. These helper cells, perhaps being more recently in contact with antigen, are effective in smaller numbers (optimum in range 10^3 to 5×10^5) than helper cells from primed mice. Such cells

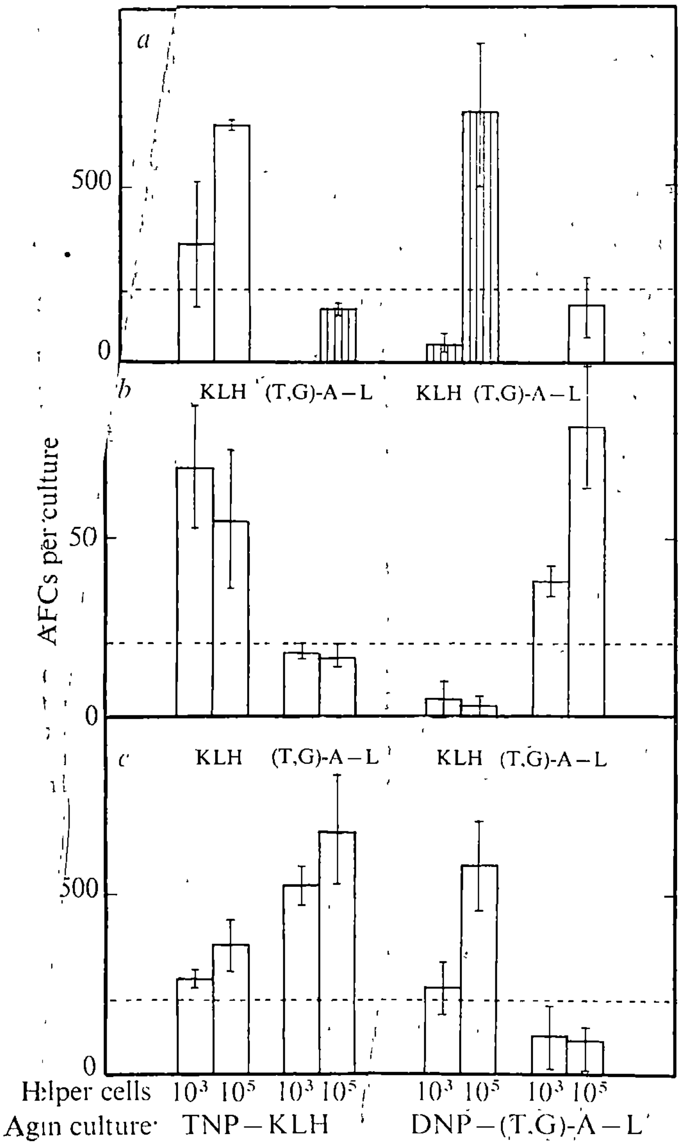
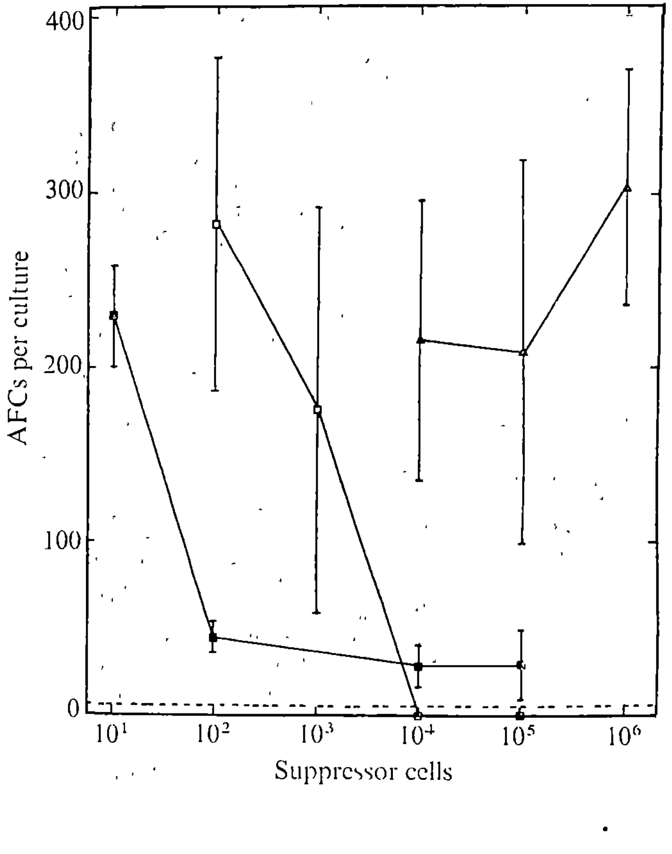


Fig. 3 Specificity of the enrichment of *in vitro* induced helper cells. Spleen cells were cultured with either KLH or (T,G)-A–L to induce helper cells. To determine the specificity of binding of helper cells to antigen-coated gels, KLH and (T,G)-A–L specific helper cells were mixed in equal numbers and applied to either KLH or (T,G)-A–L gels The adherent and non-adherent cells were tested for their helper capacity for both (T,G)-A–L and KLH by culture with spleen cells containing 1.0 μg ml^{-1} DNP–(T,G)-A–L and 0.1 μg ml^{-1} TNP–KLH respectively. Cells adherent (*b*) to KLH gels provided excellent help when challenged with TNP–KLH (for example, 700 AFC with 10^3) cells but did not help when challenged with DNP–(T,G)-A–L (180 with 10^3 cells) thus verifying the specificity of the absorption. In a reciprocal way, cells non-adherent (*c*) to KLH gels provided better help when stimulated with DNP–(T,G)-A–L than with TNP–KLH. *a*, Unfractionated cells, plated on KLH or (T,G)-A–L gels, and tested as shown Two cell doses were used (10^3 and 10^5). Two experiments of this type have yielded analogous results. Background (no helper cells added) is indicated by dotted lines.

were incubated with KLH collagen, as described (see legend to Fig. 1) and divided into adherent and non-adherent populations The adherent cells were highly efficient helper cells, with 100 cells sufficing for an optimal response (Fig. 2). This represents an enrichment factor of ~ 10^3 Repeat experiments have yielded analogous results, with the enrichment varying from 100- to 1,000-fold. The depletion of helper cells is hard to assess precisely as it is not possible to add very large numbers of cultured helper cells to the test cultures, because of the suppressive effects of these cells if added in excess of 10^6. In Fig. 2 the depletion is 100-fold. In other experiments it has ranged from 10- to 100-fold. These experiments demonstrated the capacity of the technique to enrich for KLH reactive cells, or in other experiments, chicken gamma globulin or cells reactive to (T,G)-A–L (a random copolymer of L-tyrosine, glutamic acid, coupled to DL-alanine side chains, attached to poly L-lysine backbone) have been purified. Since there was appreciable nonspecific binding (~ 0.5%) it was of importance to establish that the cells binding nonspecifically do not belong to a given subpopulation of T cells. It could be argued, for example, that helper cells (of any antigenic specificity) are preferentially retained on collagen. To exclude this possibility, and to demonstrate further the specificity of cell selection, helper cells immunised to KLH and (T,G)-A–L *in vitro* were mixed, and the mixture plated on KLH or (T,G)-A–L gels. Adherent and non-adherent cells were recovered from each, and both fractions tested with both DNP–(T,G)-A–L (for (T,G)-A–L helper cells) and TNP–KLH, for KLH helper cells. The results (Fig. 3) show that there is no detectable cross contamination of enriched helper-cell populations. Since as many as 10^5 adherent cells to one antigen, KLH, did not stimulate responses to (T,G)-A–L whereas 10^3 were sufficient for optimal help to KLH, the degree of contamination was less than 1%. Results of this type indicate that the usual nonspecific binding (~ 0.5%) is not selective for helper cells in general, and that the enrichment for helper cells is through their antigen specificity.

Another major class of T cells involved in antibody responses are suppressor cells (reviewed in ref 17). These may be specific or nonspecific. It was of interest to ascertain

Fig. 4 Enrichment of KLH-specific suppressor cells induced *in vitro*. Suppressor cells were induced by incubation of CBA mouse spleen cell with 100 μg ml^{-1} KLH for 4 d *in vitro*[30]. These cells suppress the action of KLH helper cells *in vitro* in an antigen-specific manner, and are assayed by their capacity to suppress the cooperative response of (usually) 3×10^5 *in vitro* educated helper cells and 15×10^6 normal spleen cells stimulated with 0.1 μg ml^{-1} TNP–KLH. As shown 10^4–10^5 suppressors suffice to inhibit this response After adherence to KLH gels the efficiency of these cells was augmented 100-fold, likewise the non-adherent cells were depleted of suppressor cells by 100-fold. ■, Adherent cells; □, unfractionated cells; △, non-adherent cells. Four experiments of this type have been performed with analogous results.



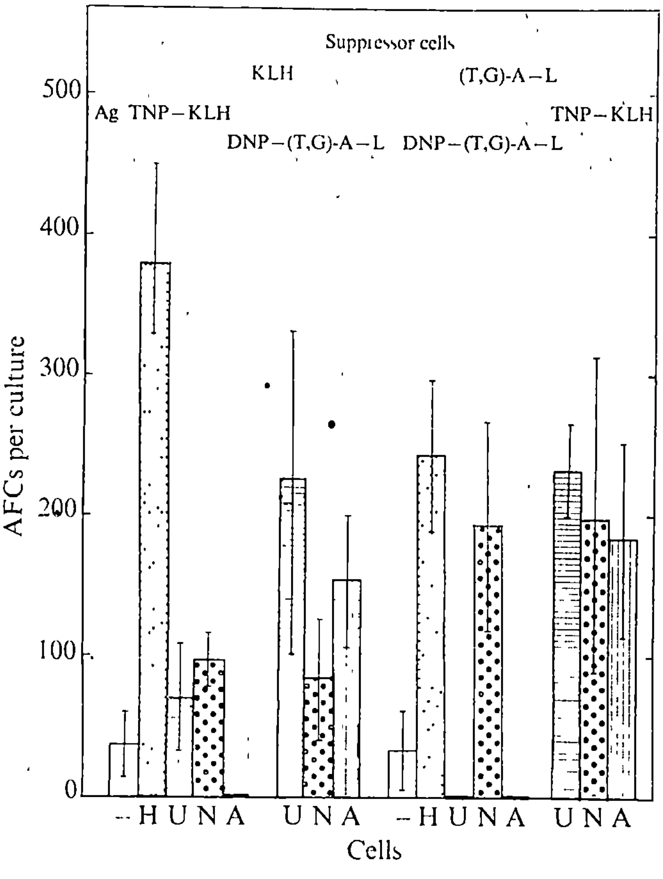


Fig. 5 Specificity of the enrichment of suppressor cells. Suppressor cells were induced *in vitro* to KLH and (T,G)-A–L. A mixture of suppressor cells were plated on KLH and (T,G)-A–L gels, and the adherent and non-adherent cells tested for their suppressor activity directed to KLH or (T,G)-A–L. As shown, KLH suppressors inhibited the response to TNP–KLH but not to DNP–(T,G)-A–L. Cells which adhered to KLH gels suppressed the response to KLH, but not to DNP–(T,G)-A–L, and vice versa. For simplicity, the results with 10^5 cells are shown. By dilution analysis the degree of specificity is > 100-fold. —, Background; H, helper cells, U, unfractionated, suppressor cells; N, non-adherent; A, adherent suppressor cells. Two experiments of this type have been performed with analogous results.

whether suppressor T cells could also be partly purified with antigen coated gels. Suppressor cells specific for KLH were obtained from cultures stimulated with a high concentration of KLH. These suppressor cells are assayed by their capacity to inhibit the response induced by KLH-specific helper cells. Figure 4 indicates that small numbers (10^5–10^4) of such suppressor cells suffice to abolish the response totally. After adherence and recovery from KLH gels, as few as 100 cells were sufficient to abolish the response, again yielding an enrichment factor of 100-fold. Repeat experiments yielded enrichments of 100–1,000-fold. The degree of depletion of suppressor cells, was, however much less satisfactory, probably because so few cells suffice for suppression. The results have varied, but a single passage on KLH gel resulted in 10–100-fold depletion, more usually nearer tenfold.

To test the specificity of the purification of suppressor cells, a mixture of KLH and (T,G)-A–L suppressor cells was purified on KLH or (T,G)-A–L gels, and the unfractionated, adherent and non-adherent (to both gels) cells were tested for suppression of the response to DNP–(T,G)-A–L or TNP–KLH. Figure 5 illustrates such an experiment, which indicates that only KLH suppressors bound to KLH, and (T,G)-A–L suppressors to (T,G)-A–L. By dilution analysis, the degree of contamination was less than 1% (full data not shown).

The results indicate that collagen is a useful substrate for the partial purification of antigen-specific T cells. It was successful with T cells primed *in vivo* or *in vitro*, with two different functional subclasses of T cells, helper cells and suppressor cells (which have recently been shown[18] to be phenotypically different).

The difficulty in demonstrating antigen-binding T cells[13,15], taken together with the increasing evidence that mouse cytotoxic T cells preferentially recognise H–2D or H–2K specificities (see for example ref. 19) and virus[20,22] or hapten modification[23], thereof, and since T cells cannot respond to antigen in the absence of macrophages[24,25], it has been suggested that, unlike B cells, T cells may only respond to antigen-modified self components, particularly transplantation antigens. Because we have succeeded in purifying helper T cells on antigen–collagen gels and this binding is blocked (by > 90%) by free antigen (to be published) it is clear that T-helper cells, at least, do have receptors for the antigen (for example KLH) *per se*. Similar considerations apply to antigen–collagen purified suppressor cells, and the results (Figs 4 and 5) together with analogous experiments performed with suppressor cell populations, in the absence of helper cells (which were killed by anti-Lyl antiserum[18]) indicate that suppressor cells can recognise antigen, and eliminate the possibility that suppressor cells can only recognise idiotypic determinants[26].

We thank Dr Edna Mozes for gifts of (T,G)-A–L, Dr Marvin Rittenberg for KLH, Dr P. Beverley for enumerating T and B cells and Mrs M. Dunkley for technical assistance. A.M. holds an EMBO long term fellowship. The authors wish to thank their colleagues for critically reading this manuscript.

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Received November 17, 1975; accepted January 30, 1976.

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Immunoglobulin-bearing cells in bone marrow of mice after prolonged treatment with anti-IgM antibodies

IN the chicken, differentiation of immunoglobulin (Ig)-bearing cells occurs in the bursa Fabricius[1,2]. Removal of the bursa at various times in the perinatal period[3-5] and/or administration of anti-μ chain antibodies have led to the postulate that IgM-bearing cells give rise in a sequential manner to cells with IgG and IgA on their surface[6]. Thus, administration of anti-μ to chicken embryos results in the elimination of cells bearing Ig of all classes, and in a depression in the synthesis of both IgM and IgG[7]. Administration of anti-μ to neonatal mice has also been shown to lead to a decrease in the serum level of all Ig classes, as well as a reduction of all Ig-bearing cells in the spleen, suggesting that the maturation sequence of Ig-bearing cells in birds and in mammals may be similar[8-10]. Recent work by one of us (D.G.O.) has shown that in mice the bone marrow is a site of production and differentiation of Ig-bearing lymphocytes, suggesting that the bone marrow may be a mammalian equivalent of the bursa of Fabricius[11-13]. The aim of the present investigation was to establish the distribution of lymphocytes bearing various Ig classes in the bone marrow of mice treated with anti-IgM, and compare it with that in normal bone marrow. The results indicate that anti-IgM injections prevent the development of lymphocytes bearing Ig of all classes in mouse bone marrow. In addition, most of the "null" small lymphocytes and many large lymphoid cells, hitherto found to be devoid of detectable surface Ig[11-14], are also eliminated.

The anti-IgM serum administered to mice was produced in rabbits by methods modified from those of Murgita *et al.*[8] and Crowle *et al*[15]. IgM was purified from the sera of BALB/c mice bearing myeloma 104E. Specific precipitates of the IgM with rabbit anti-IgM serum were injected into rabbits twice, 2 weeks apart, and were followed by a booster injection of 1 ml BALB/c serum. The animals were bled 5 d later. Serum, pooled from 64 rabbits, was twice precipitated with ammonium sulphate at 50% and 33% saturation respectively, absorbed with rat and mouse erythrocytes to remove natural red cell agglutinins, Millipore filtered, and stored in the frozen state. The final preparation had a protein concentration of 138 mg ml^{-1}; in gel diffusion in a dilution of 1 : 32 it reacted with a purified IgM preparation (Bionetics Laboratory Products), while against BALB/c serum it gave a single line of precipitation identified by immunoelectrophoresis as IgM.

The mice used in all the experiments were DBA/2 (Jackson Laboratories). One-day-old mice were injected intraperitoneally with 5 mg or 10 mg anti-IgM globulin, followed by maintenance injections of the same amount, three times weekly. A second group of experimental animals consisted of 9-week-old males, X irradiated (950 r.) and reconstituted with 2, 5 or 10×10^6 syngeneic bone marrow cells. They received 75 mg or 200 mg anti-IgM globulin 5 d later, followed by maintenance injections of 20 mg antibody globulin 3 times weekly. Controls consisted of untreated mice, or X-irradiated mice, repopulated with bone marrow, and injected with physiological saline.

Autoradiography of antiglobulin-binding cells was performed as described previously[14]. Antibody used to detect IgM-bearing cells in the first group of mice was purified by chromatography on DEAE–Sephadex from the rabbit anti-IgM pool used to inject the mice. The antisera used for the second group of animals were monospecific, anti-μ, anti-γ1, anti-γ2 and anti-α (Meloy Laboratories), precipitated with ammonium sulphate at 50% saturation. All preparations were iodinated with ^{125}I (specific activity 1.5×10^7 Ci mg^{-1}) according to the procedure of Hunter and Greenwood[16]. Femoral marrow and spleen cell suspensions were mixed at 0 °C for 30 min with the ^{125}I-antisera and washed twice through foetal calf serum gradients. The final concentration of antibody in the first preparation was 10 μg ml^{-1}; that in the commercial antisera was unknown. Smear preparations were dipped in melted Kodak NTB2 emulsion and exposed for 3 d.

Two mice injected with anti-IgM from birth were killed at 3 months of age. Autoradiographs of cells exposed to ^{125}I-anti IgM showed that 1% or less of the small lymphocytes in the spleen were IgM bearing (> 10 grains per cell) compared with 53.3% in control spleen (Table 1). In the bone marrow, IgM-bearing small lymphocytes totalled only 0.3% or less of all nucleated cells as a result of great reductions both in the total number of small lymphocytes and in the proportion showing ^{125}I-anti IgM labelling (Table 1). The small number of residual bone marrow small lymphocytes may represent mainly recirculating T lymphocytes[11-13]. The incidence in the bone marrow of large lymphoid (transitional) cells, which include the immediate precursors of small lymphocytes, was also reduced from 2.0% in the controls to 0.2% in anti-IgM-treated mice. In a separate experiment, bone marrow and spleen cells from two mice treated with anti-IgM serum for the first four weeks of life showed a similar depletion of IgM-bearing cells (mean: marrow, 0.7%; spleen, 1.1%) compared with two normal controls (mean: marrow, 39.6%; spleen, 66.4%). These results confirm the findings of a previous report, indicating depletion of Ig-bearing cells in the spleen of mice injected with anti-μ antibodies as newborn[9], and extend them to the bone marrow.

Three irradiated, marrow-reconstituted adult mice were injected with anti-IgM for 3 months and were 22 weeks of age when studied by autoradiography. The incidence of cells bearing Ig of various classes in the spleen and bone marrow of these animals and in two groups of controls is shown in Table 2.

The results in the two groups of controls are comparable, suggesting that repopulation is complete 3 months after X irradiation and bone-marrow reconstitution. The incidence of IgM-bearing cells in the spleen of these mice is rather high when compared with other reports[14,17], while the number of IgA-bearing cells is lower than reported previously[17]. These differences may be due to differences in the age and strain of mice used, or in the reagents employed. In the bone marrow, the large proportion of IgM-bearing cells agrees with previous findings[14]. The incidence of small lymphocytes bearing the other Ig heavy chains has not been reported previously in mouse bone marrow. The IgG$_2$-bearing cells in the bone marrow are clearly more numerous than could be due to immigrant cells[12,13], indicating that they are part of the indigenous population of small lymphocytes maturing in the bone marrow. This supports the view that murine bone marrow has bursa-like properties. It remains to be shown whether the smaller numbers of

Table 1 IgM-bearing small lymphocytes in spleen and bone marrow of DBA/2 mice treated with anti-IgM serum from birth

Pretreatment of mice*	Spleen small lymphocytes	Bone marrow small lymphocytes	
	IgM-bearing (%)†	IgM-bearing (%)†	Total incidence (%)‡
Saline	53.3	44.0	17 4
Anti-IgM	0.3	1.0	3.4
Anti-IgM	1.0	7.4	3.5

* Three intraperitoneal injections per week from birth, until 3 months of age.

† Small lymphocytes labelled with ^{125}I anti-IgM per 100 small lymphocytes.

‡ Small lymphocytes per 100 nucleated cells.

Table 2 Small lymphocytes bearing immunoglobulin of various heavy chain classes in spleen and bone marrow of adult DBA/2 mice, treated with anti-IgM serum

Pretreatment of mice	Spleen small lymphocytes Ig heavy chains (%)*				Bone marrow small lymphocytes Ig heavy chains (%)*				Bone marrow small lymphocytes Total incidence (%)†
	μ	γ_1	γ_2	α	μ	γ_1	γ_2	α	
Nil	69.5	5.5	38.0	6.5	46.0	5.3	26.5	4.7	25.8
X irradiation+BM+saline	63.0	6.0	37.5	8.5	47.0	5.7	29.5	6.0	21.8
X irradiation+BM+anti-IgM	0‡	0	0	0	0.3	2.0	1.0	1.0	2.9 [5.3, 2.3, 1.1]

* Labelled small lymphocytes per 100 small lymphocytes.
† Small lymphocytes per 100 nucleated cells.
‡ No labelled intact small lymphocytes were seen on extensive scan.

IgG_1 and IgA-bearing lymphocytes are also produced locally in the bone marrow, or are simply part of the recirculating lymphocyte pool.

Treatment of adult X-irradiated, bone marrow-reconstituted mice with anti-IgM resulted in the virtual elimination of all Ig-bearing small lymphocytes from the spleen and bone marrow (Table 2). As with newborn mice treated with anti-IgM, the few Ig-bearing small lymphocytes detected were pycnotic in appearance and their labelling was generally near threshold (approximately 12–15 grains, compared with heavy labelling, frequently exceeding 30 grains per cell, in controls). These experiments constitute the first successful application of anti-IgM to suppress the generation of Ig-bearing lymphocytes in adult animals. It has been reported that administration of anti-μ chain antibodies does not suppress IgM synthesis in normal adult[3], or 7-d-old mice[18]. Our results demonstrate that anti-IgM can be suppressive in the adult animal after X irradiation and bone marrow reconstitution. A full report describing the immune status of these mice will be published elsewhere (J Gordon, *et al.*, in preparation).

The mode of action of anti-IgM and the identity of the target cell are not entirely clear. Probably, anti-IgM acts primarily on early precursors of Ig-bearing cells. Supporting this view are the present observations in the bone marrow that anti-IgM treatment eliminates "null" (Ig−ve; Thy 1−ve) small lymphocytes, previously shown to be the youngest marrow small lymphocytes[11], and many large lymphocyte precursor cells. The question may be raised how anti-IgM can affect these cells on which sensitive autoradiographic techniques fail to reveal Ig[14]. The answer may be given by recent data of Melchers *et al.*[19], demonstrating a subpopulation of rapidly sedimenting marrow cells which synthesise 7–8S IgM with a considerably more rapid turnover than that of mature small lymphocytes. Conceivably, cells having such a rapid transport and release of endogenous IgM through their surface membrane may be sensitive to anti-IgM, yet apparently Ig-negative by conventional antiglobulin-binding techniques[19].

Not all types of Ig-bearing cells were eliminated by anti-IgM treatment in adult mice. In both the spleen and bone marrow, well marked antiglobulin binding was still shown by a small number of plasma cells bearing IgG_1, IgG_2 and IgA, as well as monocytoid cells, large macrophages, and damaged cells, bearing each heavy chain immunoglobulin class. Further work is required to determine the origin and significance of these cells.

The present results are consistent with the model according to which IgM-bearing cells give rise to lymphocytes carrying all other classes of immunoglobulins[6]. Murine bone marrow, like the avian bursa of Fabricius, seems to be the site of an IgM to IgG_2 sequence during lymphocyte maturation.

This work was supported by the MRC of Canada and the National Cancer Institute of Canada. We thank Miss Els Schotman and Mrs P. Young for technical assistance.

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Soluble factors substitute for T–T-cell collaboration in generation of T-killer lymphocytes

IMMUNOLOGICAL responses to histoincompatible tissues and to tumour cells are mediated by thymus-derived (T) lymphocytes. The cells that effect these responses have been termed cytotoxic T-killer cells and are derived from T-precursor cells that have become programmed (committed) to recognise specific antigenic determinants. The generation of T-effector or killer cells involves the collaboration of two distinct subpopulations of T cells. Helper T cells interact with specific precursor T cells when stimulated by antigen and, during the resultant differentiation processes, the precursor clones mature into specific killer cells[1–3]. The nature or mechanisms of the collaboration between the precursor and helper T cells has been obscure until now. The data presented here demonstrate that soluble T-cell products can replace the requirement for helper T cells in the generation of T-killer cells. The active substances are produced on antigenic stimulation and are as effective as T-helper cells during the differentiation of precursor T cells into killer cells.

Antigens of the murine major histocompatibility complex, *H-2*, were used. H-2-associated antigens served both as the stimuli for the precursor and helper T cells and as targets for the immunologically activated cells. An antigen associated with the *H-2* complex also served as a cell-surface marker for a helper

Table 1 The effects of anti-θ and anti-Ha serum treatments on responding cells in mixed lymphocyte cultures

Pretreatment of C57BL/10 responding cells*		Stimulating cells (1,000 rad)			
		None	B10.A(2R)	B10 A(5R)	B10.D2
		Average c.p.m. of ^{3}H-thymidine incorporation±s.e.			
NMS+RC		489±83	13,291±735	5,138±1,065	22,808±2,613
Anti-Ha†+RC		213±19	2,655±582	570±88	1,906±64
Anti-θ‡+RC		620±48	2,285±554	905±153	1,591±167
Anti-θ+RC—4×10^5 per ml Anti-Ha+RC—6×10^5 per ml	Mixture of pretreated cells		2,575±558	817±240	3,852±1,169
	No. of responding cells		102±4	150±13	107±12

* A total of 10^6 viable and washed cells were cultured with an equal number of stimulating cells for 98 h including an 18-h pulse with 0.5 μCi ^{3}H-thymidine per ml. Procedural details are outlined in Table 2.

† B10.A anti-B10.D2 serum plus rabbit serum as a source of complement.

‡ (AKR×RF)F_1 anti-C58 thymus cells

T-cell subpopulation. The precise *H–2* genetic region coding for the T-helper cell antigen is under investigation. The helper cell antigen is temporarily designated Ha.

The anti-helper cell serum (anti-Ha) was raised in B10.A mice against B10.D2 lymph node cells (LNCs). These congenic resistant partners differ at *H–2K* and portions of the I region of the *H–2* complex. Anti-Ha serum greatly depresses the T-cell responses of C57BL/10 LNCs to foreign H–2 antigens, *in vitro*[4] (Table 1). The effectiveness of the anti-Ha serum against the responding cell population is demonstrated by the elimination of a response to B10.A(2R)-stimulating cells (Table 1). B10.A (2R) cells are insensitive to lysis by the B10.A anti-B10.D2 serum and share *H–2K* and I-region specificities with the B10.A antibody producer[5]. Although a proportion of the cytotoxic activity to C57BL/10 cells is directed against an I-region antigen on B cells[6], depletion of non-T cells is not responsible for the depressed T-cell activity, as the mixed lymphocyte responses (MLRs) could not be restored by addition of syngeneic B cells (Table 1) in increasing numbers or of macrophages[4]. Cell-free factors produced by activated T cells could, however, reconstitute the ability to respond.

Table 2 Effect of supernatant factors on killer-cell development in cultures containing C57BL/10 cells pretreated with NMS, B10.A anti-B10.D2 (anti-Ha) serum or anti-θ serum plus complement and mixed with irradiated B10.D2 cells

Serum pretreatment of responding cells		Additions to sensitising cultures*			
		Medium	Supernatant	Medium	Supernatant
		% ^{51}Cr release†		Total killing activity‡	
Experiment *A*§	Anti-Ha+RC	17.1	52.8±2.5	11	169
	NMS+RC	48.0±3.3	58.7±2.0	145	153
Experiment *B*¶	Anti-θ+RC	11.4±0 5	11.8±0.1	1	5
	NMS+RC	55.0±0.0	55.9±1.2	248	267

* Either 1.0 ml of fresh medium or of diluted supernatant from activated C57BL/10 LNCs stimulated by B10.D2 cells was added to each culture on day 0. The final concentration of supernatant was 21% in experiment *A* and 17% in experiment *B*.

† Spontaneous ^{51}Cr release of P815-X2 target cells after 4 h was 7.7%±0.2 in experiment *A* and 10.7%±0.2 in experiment *B*. Data are presented as %±s.e.

‡ Total killing activity = % specific ^{51}Cr release × cell yield after 5 d in culture per 10^6 cells.

§ 1.0 ml of diluted serum (1:5) was added to a pellet of 15×10^6 C57BL/10 LNCs and incubated in a shaking water bath at 37 °C for 15 min. An equal volume of diluted RC (1:4) was added and the suspensions were incubated for a further 45 min Antiserum and RC were removed by centrifugation and the cells washed twice in fresh medium Equal numbers of remaining viable cells and irradiated B10.D2 cells (2×10^6 per 0.5 ml) were then cultured in RPMI 1640 medium supplemented to 0.01 M HEPES buffer, 8% human serum (heated at 56 °C for 30 min), 100 U penicillin G, 150 μg L-arginine, 2 mM L-glutamine and 75 μg kanamycin sulphate per ml.

¶ A sample of 1.0 ml of diluted serum (1:5) was added to a pellet of 4×10^7 C57BL 10 per LNCs and treated further as above before mixing with irradiated B10.D2 LNCs.

The soluble helper factor(s) was obtained through a two-step tissue culture procedure. C57BL/10 LNCs were first sensitised in culture for 4 or 5 d against irradiated B10.D2 strain LNC. The activated cells were then washed and mixed with an equal number of fresh stimulating strain LNCs. The cell mixtures (4 × 10^6 of each) were cultured in 1.0 ml of RPMI 1640 medium without serum, centrifuged at 200*g* for 5 min and incubated at 37 °C in a humidified atmosphere of 5% CO_2. Six hours later the cells were resuspended, pooled and centrifuged at 900*g* for 10 min. To ensure removal of all cells, the supernatant fluid was filtered through a 0.45-μm Millipore filter. The supernatants were either diluted and used immediately or frozen at −70 °C in the presence of 10% human serum.

The effects of the supernatant factor(s) on proliferative responses and the development of killer cells to allogeneic H–2 antigens were assessed with C57BL/10 LNCs that had been treated, before culturing, with either normal mouse serum (NMS), B10.A anti-B10.D2 serum (anti-Ha) or anti-θ serum (Thy-1.2) plus complement (RC). Rabbit serum extensively absorbed with mouse tissues served as the source of complement[7]. Anti-θ serum was raised in (AKR × RF)F_1 mice against C58 strain thymus cells. The effects of this anti-θ serum on the MLRs were reported previously[8].

The generation of cytotoxic killer T cells was greatly reduced in cultures of cells that remained after lysis with either anti-Ha serum or anti-θ serum plus RC (Table 2). The addition of the supernatant factor to the antibody-treated cells completely reversed the depressive effect of the anti-Ha serum but had no effect on killer cell development in cultures with anti-θ treated cells (Table 2).

Table 3 Effect of supernatant factors on the proliferative responses of C57BL/10 cells pretreated with either NMS, B10.A anti-B10.D2 serum (anti-Ha) or anti-θ serum plus RC

Serum pretreatment of responding cells	Supernatant concentration		
	0%	25%	6.25%
	Average c p.m. of ^{3}H-thymidine incorporation±s e.*		
Anti-Ha+RC†	1,889±636	11,495±304	10,108±53
NMS+RC†	42,353±5,859	46,494±1,594	41,069±735
Anti-θ+RC†	6,022±773	8,367±982	8,530±371

* The cultures were collected after 87 h which included a pulse of 0.5 μCi ^{3}H-thymidine per ml during the final 19 h

† The background c.p.m. of the serum treated cells were as follows: NMS+RC, 7,696; anti-Ha serum+RC, 146, and anti-θ serum+RC, 445.

C57BL/10 LNCs were treated with antiserum plus complement as outlined in Table 2. Equal numbers of viable serum-treated cells and irradiated B10.D2 cells (1.5×10^6 per 0.5 ml) were mixed 1.0 ml of either fresh medium or of diluted supernatants from C57BL/10 activated cells was then added to triplicate cultures of each combination.

Table 4 Effect of supernatant factors on the proliferative responses of non-stimulated and MLR-stimulated cell cultures

Cells cultured	Supernatant concentration*				
	0%	50%	25%	12.5%	6.25%
	(average c.p.m. of ^{3}H-thymidine incorporation±s.e.)†				
C57BL/10	2,253±33	3,240±571	2,585±187	3,473±446	2,325±123
C57BL/10+B10.D2 (1,000 rad)	11,852±1,852	15,581±549	18,749±1,092	15,341±1,415	10,065±1,059
B10.D2+C57BL/10 (1,000 rad)	8,875±1,883	18,148±3,610	13,897±1,069	16,015±1,069	5,814±1,264

* The supernatant used in these cultures was produced by C57BL/10 cells after activation with B10 D2 irradiated cells for 5 d.
† The cultures were collected after 66 h which included a pulse of 0.5 μCi ^{3}H-thymidine per ml during the final 18 h of culture.

Three conclusions can be drawn from these data. First, the C57BL/10 precursor T cells that respond to H–2 antigens are not lysed by the anti-Ha serum plus RC. We have also demonstrated that the depressive effect of this antiserum on MLR cannot be reversed by the addition of various numbers of cells remaining after lysis with anti-θ serum plus RC[4] (Table 1). Therefore, the depleted H–2 responsive cell must reside in the θ-antigen-bearing T-cell population but not, as demonstrated in Table 2, in the precursor subpopulation. The most likely target of the B10.A anti-B10.D2 serum, then, is the helper T cell.

Second, the supernatant factor(s) can serve functionally as an adequate substitute for the antibody-depleted cells in the generation of killer T cells. Although much information has been accumulated about T-cell replacement factors in B-cell-mediated responses[9–18], this is the first evidence that a stable soluble helper product mediates T–T-cell collaboration.

Third, one of the functions of the helper factor(s) is to increase the numbers of killer cells generated. The total yield of killer cell activity obtained from C57BL/10 cells treated with the anti-Ha serum and subsequently cultured with stimulating cells in the presence of the supernatant factor(s) was equivalent to that obtained with NMS-treated cells (Table 2). This increase in killing activity was probably accomplished through the enhancement of clonal proliferation of the precursor cells. Clearly, the helper factor provided a signal that was necessary for DNA synthesis (Table 3). The helper factor was active even at quite dilute concentrations, in the enhancement of the MLRs obtained with cells remaining after lysis with anti-Ha serum (Table 3). The helper factor(s) had only slight effects on the proliferative responses of cultures that either contained both (NMS + RC) or lacked both (anti-θ + RC) the precursor and helper T-cell subpopulations (Table 3). Similar results were obtained with C57BL/10 LNCs cultured without irradiated stimulating cells (Table 4). Enhanced proliferation of unsensitised cells was never greater than 2.5-fold over background in three separate experiments. The helper factor did enhance MLR-induced proliferation within a wide range of concentrations and apparently was not specific for the stimulating H–2 antigens (Table 4).

The difference between the levels of thymidine incorporated and the total yield of cytotoxicity generated in supernatant-supplemented cultures of cells remaining after lysis by anti-Ha serum is revealing. In the normal MLR, helper cells as well as precursors proliferate, thus both subpopulations contribute to the observed responses. The proliferative contribution of helper cells is lost, however, in cultures pretreated with anti-Ha serum. At the levels of supernatant tested, maximum ^{3}H-thymidine incorporation by the antiserum-resistant precursor cells is reached even at the lower concentrations (Table 3). The total yield of killer-cell activity in the supernatant-supplemented cultures of anti-Ha-treated cells is equivalent to that obtained with unfractionated T–T-cells (Table 2). Thus the sequence of events in the generation of T-effector cells seems to be: the release of soluble factors and proliferation of helper cells on contract with antigen and the subsequent stimulation of proliferation and potentiation of specific precursor cells by the helper factor and antigen.

The relationship between the helper factor described here and the T-cell replacement factors that function in mediating B-cell responses is being investigated. Many of the factors that help in antibody production have been obtained from allogeneic cell cultures. The nonspecific allogeneic effect factor[17] and the specific T-cell factor of Munro and Taussig[18] which mediate B-cell responses are of particular interest as each can be removed with anti-H–2 serum-conjugated immunoabsorbants. It is possible that the H–2-associated marker detected on the helper T cells is similar or identical to a determinant on the soluble helper substance probably produced by these cells. This helper cell marker is expressed by a small proportion of T-effector cells and the end-stage or mature killer cells may no longer express the antigen[4]. This supports the notion that the H–2-associated helper cell marker is also expressed by the soluble helper product and that it functions in the differentiation of the precursor T cells to effector T cells.

I thank B. Allitto, B. Malynn and C. Pinto for technical assistance and Dr D. B. Amos for critical evaluation of the manuscript. This work was supported by a grant from the National Cancer Institute.

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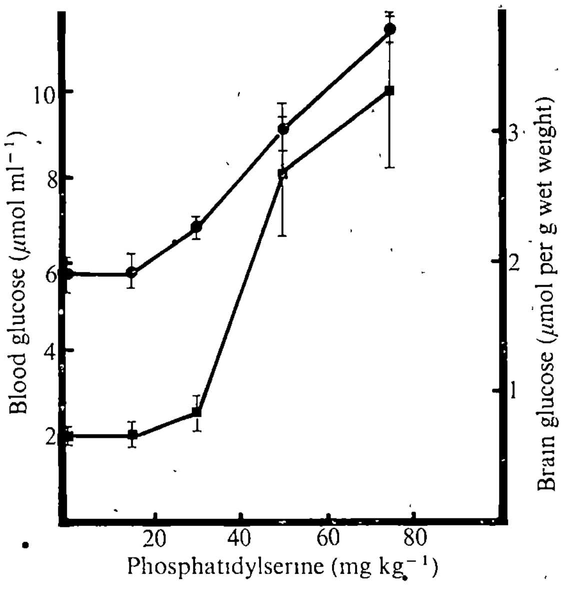
Pharmacological effects of phosphatidylserine liposomes

PHARMACOLOGICAL studies on diacylphospholipids develop along two lines of investigation. In the first, preparations of liposomes are used as carriers of drugs either bound to the bilayer or entrapped inside the vesicles. Sonicated liposomes have been shown to be able to transport inhibitors or drugs to target sites *in vitro*[1] and *in vivo*[2]. In the second, liposome preparations are administered and pharmacological effects are recorded. In this context significant effects have been observed on cholesterol distribution[5]. The molecular mechanism underlying the possibility of pharmacological effects *in vivo* by long chain diacylphospholipids is indicated by a series of investigations[4–7] showing the capacity of phospholipid vesicles to fuse with cellular membrane *in vitro*. As a consequence of changed composition of membrane lipids, alteration in the permeability properties, transport systems and other functions of biological membranes are expected to occur. It has been shown[8] that sonicated dispersion of bovine brain phospholipids can induce in mice modification of glucose distribution Purification of lipid mixtures indicated that phosphatidylserine was one of the active components. Phosphatidylserine liposomes have shown great capability to interact *in vitro* with biologically active compounds (histamine[9] or calcium[10]), with phospholipid-dependent enzymatic systems (Na$^+$–K$^+$-stimulated ATPase[11] or tyrosine hydroxylase[12]), or with intact cells (enhancement of histamine release during antigen–antibody interaction[13]; promotion of cell fusion[4]). In this investigation data on phosphatidylserine-induced effects *in vivo* are presented.

Fed male albino mice were used throughout. The purity of phosphatidylserine extracted from bovine brain[14] was checked by thin-layer chromatography using Silica gel G and a solvent of chloroform–methanol–water (65 : 35 : 4, by volume). Dispersion of phosphatidylserine was obtained by 8-min sonication (MSE apparatus) at 0 °C in 50 mM Tris-HCl, *p*H 7.4. Liposomes in 0.1–0.2 ml buffer were injected intravenously in the tail vein within a few hours of their preparation, and the animals killed 30 min later by decapitation so as to collect the blood and allow the head to fall into liquid nitrogen. Brain hemispheres were removed, powdered under liquid nitrogen together with frozen 0.5 ml 0.3 N perchloric acid and allowed to warm at 0–4 °C. Glucose was estimated by the hexokinase method on perchloric acid extract[15].

Figure 1 shows the effect of phosphatidylserine liposomes on free glucose content of blood and brain tissue. The level of glucose was increased in both compartments; 15–30 mg kg^{-1} were required to trigger the effects (0.35–0.70 mg per mouse, equal to about 0.5–1.0 µmol phosphatidylserine) This low amount, which can be calculated to be composed of about 2×10^{14} vesicles[4], is not expected to produce gross changes in the body circulation. Accordingly, variations in brain glycogen or lactate were not recorded. Parallel experiments showed that neither uncharged liposomes of phosphatidylcholine nor charged, small liposomes of bovine brain phosphatidylinositol produced effects on glucose distribution. Among phosphatidylserines, liposomes made by purified soya bean phosphatidylserines were only slightly or not active, suggesting a role of both acyl chain and structure of polar head. As it was observed with the mixture of bovine brain phospholipids[8], the effect of phosphatidylserine liposomes was more manifest after intravenous injection and dispersion by sonication at neutral *p*H (Table 1) The enhancement of brain–blood glucose ratio revealed the tendency for glucose accumulation in the brain tissue. The need for small liposome formation in order to produce the pharmacological effect is difficult to explain in terms of possibility to cross membrane barriers. The average diameter of phosphatidylserine liposomes prepared by sonication[16] is estimated to be about 200 Å, not compatible with free transfer across cellular membranes, the blood–brain

Fig. 1 Effect of increasing amounts of phosphatidylserine on glucose distribution in mice. Sonicated dispersion of liposomes in 50 mM Tris-HCl (*p*H 7.4) were injected intravenously to fed mice which were killed 30 min later. Other conditions described in text. Values are mean ± s.e. of four animals. ●, Blood; ■, brain.



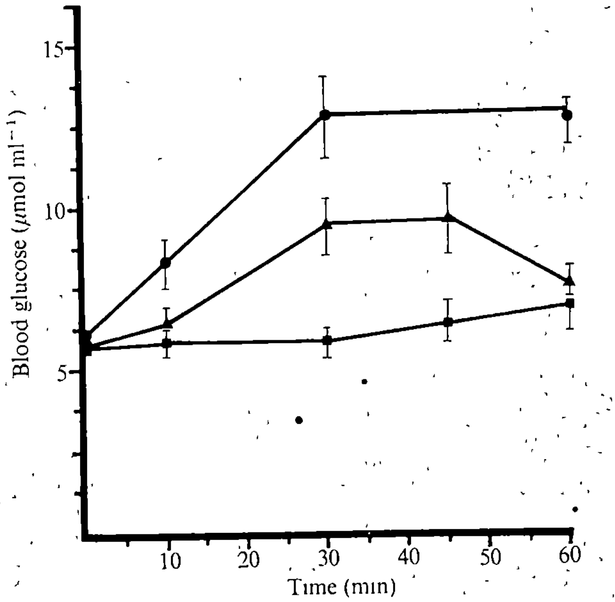


Fig. 2 Antagonism by Hydergine (dihydroergotoxine): sonicated liposomes (75 mg kg^{-1}) containing phosphatidylserine were given intravenously to mice treated (▲) or not (●) with hydergine (2 mg kg^{-1}) 30 min before phospholipid injection. Mean ± s.e. of four animals. ■, Hydergine alone.

Fig. 3 Decrease in adrenal catecholamine content. Mice were injected intravenously with sonicated liposomes of phosphatidylserine (150 mg kg^{-1}). At the time indicated the animals were killed and the adrenals homogenised in 0.4 N perchloric acid containing 0.1% sodium metabisulphite. Total catecholamines determined[18] on about 2 mg tissue. Values are mean ± s.e. of separate determinations whose number is given in parentheses.

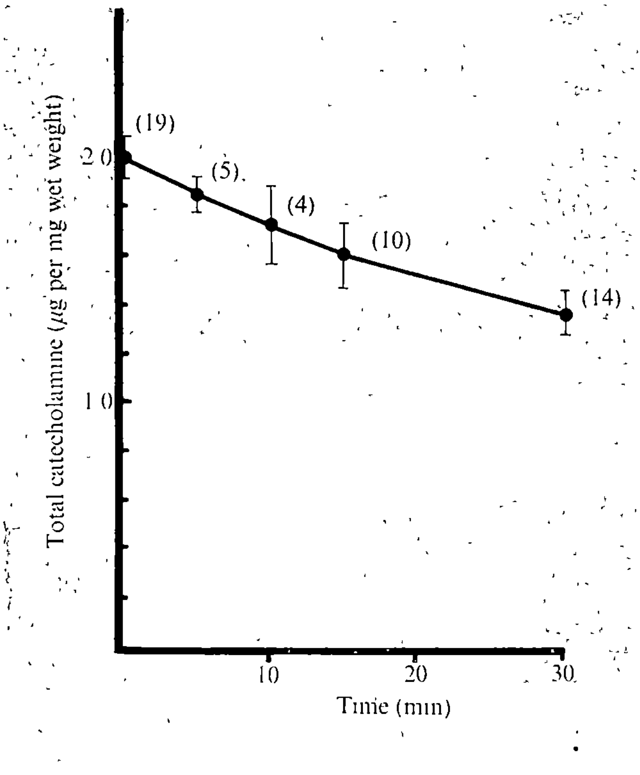


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Table 1 Effect of phospholipid dispersion

Liposomes dispersed by		Administration	Blood glucose (μmol ml^{-1})	Brain glucose (μmol per g wet weight)	Brain–blood ratio
—	(17)	—	6.21±0.13	1.33±0.05	0.21
Homogenisation	(5)	Intraperitoneal	6.76±0.67	1.44±0.05	0.21
Sonication	(5)	Intraperitoneal	7 45±0.59	1.62±0.05*	0.22
Homogenisation	(5)	Intravenous	7.35±0.79	1.55±0.18	0.21
Sonication	(17)	Intravenous	10.17±0.40*	3.67±0.23*	0.36

*$P < 0.001$.

Liposomes were injected into fed mice at 75 mg phosphatidylserine per kg. Dry phospholipid was resuspended in 50 mM Tris-HCl buffer and dispersed by simple homogenisation or sonication (see text). Number of animals given in parentheses. Values are mean ± s.e. Significance levels (P) were assessed using Student's t test.

barrier or even the capillary wall of many organs. The effect of sonication is better understood in terms of facilitation of lipid–lipid, lipid–protein interaction or binding of blood cations. Penetration of liposomes into parenchymal cells is also enhanced after reduction of their size by sonication[17]. This would enable liposomes to produce a direct influence on cell carbohydrate metabolism in some organs (for example, liver). In spite of its high activity, the phosphatidylserine content does not completely account for the effect of bovine-brain phospholipid mixture observed earlier[8]. It is possible that other active components are present or that the optimal ratio between phosphatidylserine and other phospholipids increased the effect A mixture of phosphatidylcholine, lysophosphatidylcholine and phosphatidylserine was more active than phosphatidylserine alone in inducing cell fusion[4].

The involvement of catecholamine release in the hyperglycaemic effect is indicated in the experiments of Fig. 2 in which it is shown that previous administration of the adrenolytic agent Hydergine (dihydroergotoxine) strongly reduced the phosphatidylserine-induced increase of blood glucose level In separate experiments it was found that in the Hydergine-treated mice the normal values of glycaemia did not prevent the effect of liposomes on brain glucose, in agreement with the observations on lipid mixtures[8]. Attempts were made to demonstrate a decreased level of catecholamine content in the adrenal glands after phosphatidylserine injection. Figure 3 shows that a reproducible reduction of about 30% was found 30 min after administration of phospholipid vesicles (150 mg kg^{-1}). The increased cerebral content of glucose, the lack of lactate accumulation and the ineffectiveness of phosphatidylcholine and phosphatidylinositol excluded the possibility that nonspecific damage of blood circulation followed by cerebral hypoxia was responsible for catecholamine release.

The accumulation of free glucose in brain tissue and the release of catecholamines are phosphatidylserine-induced pharmacological effects which can be differentiated by the use of an adrenolytic agent. In spite of this distinction, a correlation between the two effects seems likely and it is indicated by the observation of a dopamine-induced high level of brain glucose[19]. The same pattern of activity (ineffectiveness of phosphatidylcholine and phosphatidylinositol, high activity of phosphatidylserine) was found on histamine release *in vitro*[20], an effect which required calcium. Calcium bridges between the negatively charged cell surface and liposomes may be required for the attachment of vesicles, as was suggested for phosphatidylserine-induced fusion of cultured cells[4]. This indicates a general capacity of phosphatidylserine vesicles to promote *in vivo* and *in vitro* the calcium-dependent release of cellular materials stored in specialised structure. Extending these observations it was found that injection of sonicated liposomes of bovine brain phospholipids increased catecholamine metabolism in mouse brain[21] and produced release of acetylcholine from rat brain cortex[22]. Phosphatidylserine was the active component in both cases. Further investigations are required to establish whether these effects taking place in the central nervous system reflect liposome penetration into the brain fluids.

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Received December 1, 1975; accepted January 21, 1976.

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Bacterial membrane transport of β-exotoxin, an anti-metabolite of RNA synthesis

β-Exotoxin is produced extracellularly by certain strains of *Bacillus thuringiensis* during the stationary growth phase, apparently resulting from nucleic acid metabolism[1]. It is a monophosphorylated adenine nucleoside derivative[2,3] and can modify RNA polymerase activity of mammalian and bacterial origin *in vitro* through substrate competitive inhibition[4,5]. RNA polymerase in nuclei from rat liver[6] and insect larval fat bodies[7] is sensitive to exotoxin, and inhibition seems to be subject to reversal by ATP. Other functional enzymes that catalyse reactions involving ribonucleoside triphosphates are similarly inhibited by exotoxin, such as adenylate cyclase[8]. Exotoxin is more toxic in mammalian and other eukaryotic systems than in cell-free prokaryotic extracts, and it can cross cell and nuclear membranes in mammalian systems[5,8,9]. But I know of no report of any effect of exotoxin on intact, physiologically normal bacterial cells. Therefore I have investigated the intracellular effect of exotoxin on RNA synthesis in actively metabolising bacterial cultures. This report describes the intracellular inhibition of RNA synthesis by exotoxin in cultures of *Escherichia coli* and *Bacillus subtilis* treated to increase their permeability. Further data revealed that exotoxin is totally excluded by the bacterial membrane, even in those treated cells.

Both organisms were impermeable to exotoxin in normal conditions. Extracellular exotoxin concentrations up to 1 mg ml^{-1} caused only slight inhibition (5% or less) of RNA synthesis in exponential cultures of *B. subtilis* and *E. coli*. Initially, no methods designed to increase membrane permeability of exponentially growing cells[10-13] increased exotoxin inhibition of cellular RNA synthesis. An adaptation of one method, however, involving EDTA–Tris treatment followed by resuspension of the washed cell pellet in complete minimal medium at *p*H 5.2, gave greater sensitivity to exotoxin. The rate of RNA synthesis was near normal in these cultures, although a lag of 2–3 h occurred before replication resumed.

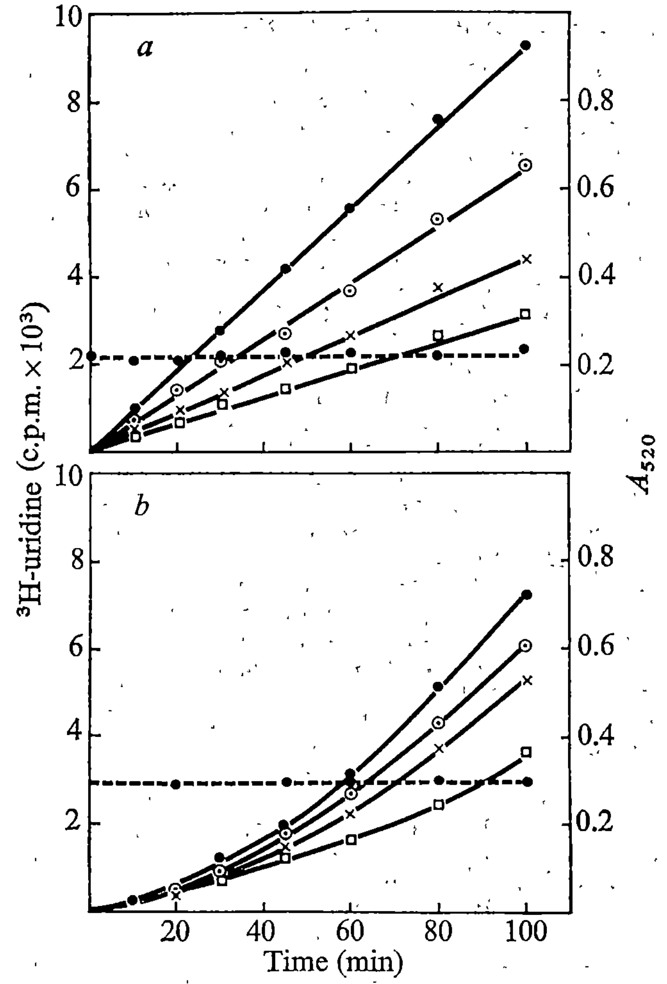

Fig. 1 *In vivo* inhibition of RNA synthesis by exotoxin. *a*, *E. coli*; *b*, *B. subtilis*. Cultures were pretreated by the EDTA–*p*H shift method. Extracellular exotoxin concentration: ●, 0 μg ml^{-1}; ○, 50 μg ml^{-1}; ×, 100 μg ml^{-1}; and □, 500 μg ml^{-1}. The absorbance of an uninhibited control culture (without exotoxin) is also shown (- - - -). Cultures of *E. coli* and *B. subtilis* were grown on a minimal salts medium at *p*H 6.8 supplemented with 0.2% glucose; filter-sterilised tryptophan (40 μg ml^{-1}) was added for growth of the *Bacillus* culture. Mid-exponential phase cultures were treated to increase their permeability by Leive's method[10]. Treatment was for 2 min (25 °C) with 0.25 M EDTA, after which the culture was diluted with 0.125 M Tris-HCl, centrifuged, washed again with Tris-HCl, recentrifuged, and the pellet resuspended in complete minimal medium at *p*H 5.2. Measurement of isotope incorporation in these permeabilised cells was determined by incubation on a water bath shaker (30 °C) in the presence of ^{3}H-uridine (specific activity, 3.7 μCi μmol^{-1}) and various concentrations of exotoxin by withdrawing 1-ml samples at intervals and by precipitating the RNA with cold 10% TCA. The samples were filtered on Whatman GF/A glass-fibre filters, washed with TCA (containing 0.4 mM carrier uridine) and cold ethanol, dried, and counted by liquid scintillation.

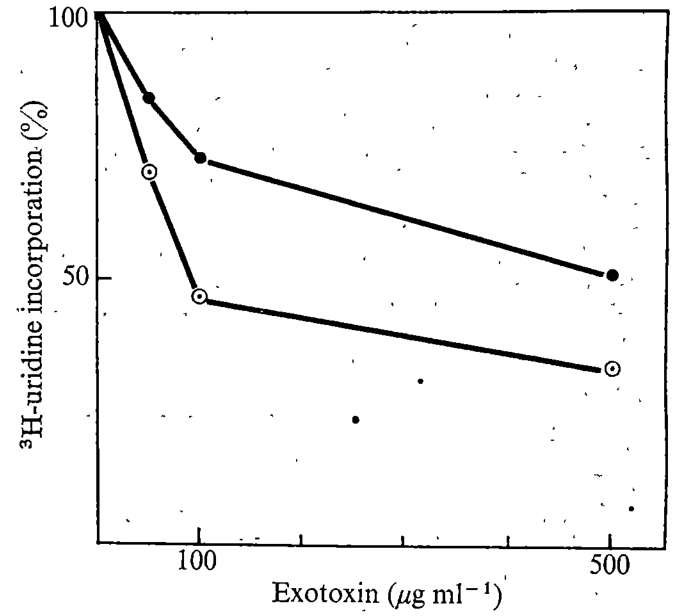

Fig. 2 Percentage inhibition of ^{3}H-uridine incorporation by exotoxin. ○, *E. coli*; ●, *B. subtilis*. Values are percentage incorporation compared with an uninhibited control after 100 min incubation, and reflect the final data points from Fig. 1 for both organisms.

Incorporation of ^{3}H-uridine into trichloroacetic acid (TCA)-precipitable RNA in the presence and absence of exotoxin is shown in Fig. 1. Total RNA synthesis after 100 min in *E. coli* cells treated with EDTA–Tris was inhibited 29.9, 53.2 and 66.1%, respectively, by the addition of exotoxin at 50, 100 and 500 μg ml^{-1}. Corresponding data were obtained from *B. subtilis* cells pretreated similarly; inhibition of total RNA synthesis after 100 min was 16.3, 23.1 and 49.2%, respectively, in the presence of the same concentrations of exotoxin. No cell division occurred with either EDTA–Tris-treated culture during the measured interval of uridine incorporation.

The degree of inhibition at each concentration of exotoxin fluctuated little throughout the experiment with both organisms. The inhibitory response, however, was not linear with respect to increased exotoxin concentration, but became less sensitive above 100 μg ml^{-1}, or 1.25×10^{-4} M (Fig. 2). These results corroborate data for *in vitro* exotoxin inhibition for purified *E. coli* RNA polymerase[14].

Attempts to measure exotoxin transport into EDTA–Tris-treated cells (not *p*H shifted) of either organism failed. The uptake of ^{3}H-uridine and ^{3}H-exotoxin was measured in the presence of rifampin so that only membrane transport would be measured and incorporation could be excluded. The effect of unlabelled exotoxin on ^{3}H-uridine uptake was followed. Exotoxin did not penetrate the membrane of EDTA–Tris-treated cells of either *E. coli* (Fig. 3) or *B. subtilis*. Uridine transport was most rapid during the first 5–10 min in both organisms, and then it diminished to a plateau when an inhibitor of RNA synthesis was present. Unlabelled exotoxin had little effect on uridine transport of *B. subtilis*, but slightly reduced uridine transport in *E. coli*. Growth was resumed in EDTA–Tris-treated cultures approximately 20 min after treatment.

Historically, bacterial membranes have been known to be poorly permeable to hydrophilic compounds. Nucleotides and hexose phosphates are classic examples. EDTA treatment disrupts the outer membrane of Gram-negative bacteria and provides improved access for some compounds, yet the inner membrane remains intact and presents still another barrier for certain external molecules[15]. Whereas deoxyribonucleoside triphosphates gain rapid entrance inside permeabilised cells of *E. coli*, the corresponding monophosphates are almost totally excluded[16]. The exotoxin produced by *B. thuringiensis* is structurally similar to deoxyribonucleoside monophosphates, and its inhibitory activity toward RNA polymerase is based on substrate analogue competition in cell-free extracts. My results, however, suggest that whatever the effect of exotoxin on cellular RNA synthesis in bacteria, it occurs externally. Figure 3 indicates that exotoxin may depress uridine uptake in *E. coli*, which could interfere with normal internal ribonucleotide precursor pools. Comparable experiments with cells of *B. subtilis* were inconclusive.

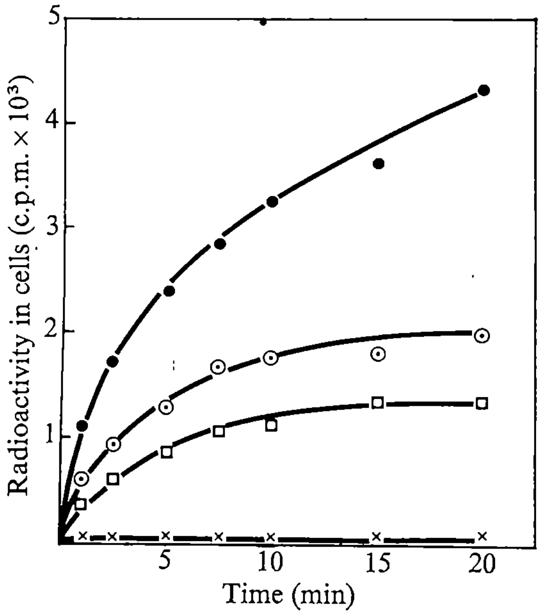

Fig. 3 Uptake of ^{3}H-uridine and ^{3}H-exotoxin in cells of *E. coli* treated with EDTA-Tris. ^{3}H-uridine uptake: ●, without rifampin; ○, with rifampin (50 μg ml^{-1}); □, with rifampin and unlabelled exotoxin (100 μg ml^{-1}). ×, ^{3}H-exotoxin uptake. Transport was measured with EDTA-Tris-treated cells, which were resuspended in complete minimal medium at *p*H 6.8 and held on ice until used. Either tritium-labelled uridine (Fig. 1) or exotoxin (specific activity about 0.27 μCi μmol^{-1}) was added to samples of permeabilised cells and placed in a reciprocating water bath shaker at 18 °C. Rifampin was added (50 μg ml^{-1}) to prevent *in vivo* ^{3}H-uridine incorporation into RNA Samples (1 ml) containing whole cells were withdrawn at intervals and immediately filtered on GF/A filters, washed with cold buffer (10 mM Tris-HCl, 50 mM KCl, *p*H 7.9), dried, and counted by liquid scintillation. Both labelled and unlabelled exotoxin were provided by T. R. Shieh (International Minerals and Chemical Corporation).

B. thuringiensis exotoxin has little or no effect on RNA synthesis in untreated cultures of *E. coli* and *B. subtilis*. Even in specially treated cultures with increased permeability, exotoxin seems to be totally excluded by the bacterial membrane. But RNA synthesis in similarly treated cultures of these organisms is inhibited by exotoxin in a concentration-dependent order. Thus inhibition of RNA synthesis by exotoxin in intact bacteria seems to be linked to membrane transport and not to competitive inhibition of RNA polymerase.

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Received December 12, 1975, accepted January 12, 1976.

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Role of mitochondria in control of calcium content of liver slices

INTRACELLULAR Ca^{2+} is thought to be important in the control of metabolic activity[1,2]. In liver cells, three energy-dependent transport activities capable of modifying the cellular distribution of this ion have been described—its accumulation by mitochondria[3,4] and endoplasmic reticulum[5] and its extrusion from the cell[6,7]. Mutual interactions, however, between these activities and their relative importance in determining total cell Ca^{2+} have so far been little studied in intact cells. One reason for this has been the difficulty of avoiding redistribution of Ca^{2+} between organelles when the cells are broken for isolation procedures, although Chen *et al*[8] used ruthenium red, added to the homogenisation medium, to prevent such redistribution in crab hepatopancreas. We now show that redistribution of Ca^{2+} into the mitochondria of rat liver can also be largely prevented by adding La^{3+} (another inhibitor of mitochondrial Ca^{2+} transport; ref. 9) to the homogenisation medium. We have also studied the contribution of mitochondria to the Ca^{2+} content of rat liver slices incubated in conditions in which total cell Ca^{2+} varies greatly.

Slices of rat liver were prepared and incubated as described previously[7]. Mitochondria were isolated from fresh liver and from incubated slices[10] using medium containing: mannitol 250 mM, sucrose 75 mM, EDTA 0.1 mM and Tris-HCl (*p*H 7.4) 2.5 mM. The swollen mitochondria of the 'fluffy layer' occurred in greater quantities in incubated slices than in fresh liver[11]; as we wished to determine the total mitochondrial contribution to the tissue Ca^{2+}, we usually analysed the mitochondrial pellet and 'fluffy layer' together Mitochondria from all preparations showed coupled, oligomycin-sensitive respiration[11]. For analysis, samples of slices and tissue fractions were dried and ions (including ^{45}Ca) extracted from the dry tissue[7]. Total Ca^{2+} was determined by atomic absorption spectrophotometry[7] and ^{45}Ca by standard techniques of scintillation counting. Protein was determined by a Biuret method[12]

To study the effectiveness of adding La^{3+} to the homogenisation medium as a means for preventing the redistribution of Ca^{2+}, we measured the entry of ^{45}Ca into mitochondria isolated from liver slices that had been incubated for 90 min at 1 °C. In these conditions the slices show the highest total Ca^{2+} content (see below). To half of the slices, ^{45}Ca was added in the homogeniser tube only 5 s before their disruption, so that the isotope could be considered totally 'exogenous'. In this case, the ^{45}Ca and La^{3+} present in the homogenisation medium would have reached the mitochondria simultaneously and a maximal possible inhibition of ^{45}Ca entry would be expected. Table 1 shows that about 50% of the added ^{45}Ca was taken up by the control mitochondria (homogenised without La^{3+}), and that the presence of 1 mM La^{3+} inhibited this uptake by 90%. Thus, La^{3+} can be used effectively to block redistribution of tissue Ca^{2+} into the mitochondria on

Table 1 Effect of presence of La^{3+} during homogenisation on entry of ^{45}Ca into mitochondria isolated from liver slices

^{45}Ca added	Slice group	La^{3+} added to homogeniser (mM)	^{45}Ca in mitochondria (10^{-6} × d.p.m. per g protein)	Total Ca^{2+} in mitochondria (mmol per kg protein)
To homogeniser	*a*	0	35.8±4.0*	—
	b	1	3.9±0.5	—
To incubating slices	*c*	0	1.49±0.08†	63.2±2.1
	d	1	0.68±0.05	47.3±1.7

* 51.2±3.5% of radioactivity in whole homogenate.
† 60.9±3.8% of radioactivity in whole homogenate.

Slices were incubated for 90 min at 1 °C in a Ringer solution containing, 146 mM Na$^+$, 5 mM K$^+$, 1.3 mM Ca^{2+}, 1.0 mM Mg^{2+}, 161 mM Cl$^-$, 1.0 mM SO$_4{}^{2-}$ and 10 mM Tris (*p*H 7.4). After incubation the slices were separated from the medium by suction and washed once with the mitochondrial isolation medium to remove superficial ions. The slices were then rapidly transferred to the isolation medium. The slices were divided into four groups which were treated as follows: *a*, ^{45}Ca was added to the isolation medium in the homogeniser, to give 0.5 μCi ml^{-1} (0.5 μCi per μmol Ca^{2+}), 5 s before homogenisation of the slices. *b*, As for group *a*, but with 1 mM La^{3+} present in the homogenisation medium. *c*, ^{45}Ca was added to the Ringer solution containing the slices after incubation at 1 °C for 89 min, giving 0.5 μCi ml^{-1} (0.38 μCi per μmol Ca^{2+}). *d*, As for group *c*, but with 1 mM La^{3+} present in the homogenisation medium. The medium used for rehomogenisation of the 600*g* pellets (ref. 9) of groups *b* and *d* contained 0.5 mM La^{3+}; further steps in the isolation procedure were carried out in medium free of La^{3+}. The counting rate for ^{45}Ca was corrected for background, and for quenching by the channels-ratio method; counting efficiency was approximately 55%. The results reported are mean ± s.e. of the mean from four experiments, each analysed in triplicate.

homogenisation. In the remaining slices we determined the effect of La^{3+} present during homogenisation on the distribution of ^{45}Ca added 1 min before the end of incubation at 1 °C (and approximately 1.5 min before homogenisation). The slices were rinsed free of the superficial, labelled Ringer solution before homogenisation so that all the remaining ^{45}Ca in the slices could be considered 'endogenous'. Mitochondria isolated from these slices without added La^{3+} again contained much of the total ^{45}Ca in the homogenate, but the presence of 1 mM La^{3+} reduced the recovery of isotope in the mitochondria by about 55%. We conclude that this inhibition largely represents the prevention of Ca^{2+} entry into the mitochondria on disruption of the tissue, and that the 45% of the ^{45}Ca still found in the mitochondria had entered by exchange before homogenisation.

It follows that the extent to which cell Ca^{2+} undergoes redistribution into the mitochondria on homogenisation should be indicated by a smaller Ca^{2+} content of organelles isolated in the presence of La^{3+}. Table 1 shows that slices homogenised with 1 mM La^{3+} yielded mitochondria containing 25% less Ca^{2+} than those homogenised in its absence. In a further series of experiments, La^{3+} at concentrations of 0.1, 0.5 and 2.0 mM reduced the mitochondrial Ca^{2+} from a control value of 47±5 mmol per kg protein to 42±3, 42±2 and 38±2 mmol per kg protein, respectively—that is, a fall of 20% at 2 mM La^{3+}. We concluded that the maximal effect of La^{3+} was at 1 mM, and that 20–25% of the Ca^{2+} of mitochondria isolated in the absence of La^{3+} was derived from extramitochondrial sources on homogenisation. La can precipitate tissue proteins[13] and when the ratio of La^{3+} to slice tissue in the homogeniser was 24 μmol per g wet weight we found a reduction of 50% in the recovery of mitochondrial protein. The protein yield, however, was not reduced when the ratio was 8–12 μmol La^{3+} per g wet weight, and this range was used in all the experiments we describe here.

The contribution of mitochondria to the total Ca^{2+} content of liver slices incubated in a Tris-buffered Ringer solution containing 1.3 mM Ca^{2+} was next determined in various conditions, subsequent homogenisation being carried out in the presence of 1 mM La^{3+}. The slices underwent a threefold increase in Ca^{2+} content at 1 °C, and much of the extra Ca^{2+} was extruded during subsequent incubation at 38 °C (Table 2). The mitochondrial Ca^{2+} changed in parallel with the total tissue content, showing a sixfold increase at 1 °C and a subsequent recovery towards low Ca^{2+} levels at 38 °C, which was even more complete than that of the whole slice. Whereas the mitochondria were calculated to contribute 35% of the total Ca^{2+} of the slices at 1 °C, they only contributed 17% after recovery (Table 2). Preliminary experiments in which La^{3+} had not been used during homogenisation, showed qualitatively similar results whether slices were incubated in Tris-buffered or phosphate-buffered Ringer solutions[14]. In a few experiments the 'fluffy layer' was isolated and analysed separately; it was found to undergo qualitatively similar changes but with a Ca^{2+} content which was about 30% less, per unit protein, than the mitochondrial pellet proper. In contrast to the parallel changes of whole-slice and mitochondrial Ca^{2+} just described, the partial (35%) inhibition by cyanide of Ca^{2+} extrusion from the slices was not reflected in any inhibition of the loss of Ca^{2+} from the mitochondria (Table 2).

Mitochondrial K$^+$ participated in the overall loss of this

Table 2 Ca^{2+} and K$^+$ contents of liver slices and of mitochondria isolated from them after incubation in varying conditions

	Fresh tissue	90 min at 1 °C	90 min at 1 °C followed by 30 min at 38 °C Control	Cyanide (2 mM)
Ca^{2+}				
Intact slices (mmol per kg slice dry weight)	4.4±0.8	12.8±1.5	8.4±0.8	10.0±1.7
Mitochondria (mmol per kg protein)	7.3±0.6	44.1±3.7	11.9±0.9	11.8±2.5
(mmol per kg slice dry weight)	1.1	4.5	1.4	1.4
K$^+$				
Intact slices (mmol per kg slice dry weight)	317±20	103±6	173±11	88±5
Mitochondria (mmol per kg protein)	147±3	91±2	84±4	37±3
(mmol per kg slice dry weight)	21	9	10	4

Slices were incubated in Tris-buffered Ringer solution (see Table 1) as indicated, the vessels being gassed with O$_2$ before incubation at 38 °C. Samples of the slices were taken for analysis after incubation; the remainder were homogenised in the presence of 1 mM La^{3+} and their mitochondria isolated together with the 'fluffy layer'. The quantities of intramitochondrial ions are expressed per kg mitochondrial protein and per kg tissue dry weight, the latter being calculated from the recovery of mitochondrial protein per unit of slice dry weight. The results shown are mean ± s.e. of the mean from three experiments, each carried out in triplicate.

ion from slices incubated at 1 °C and the further loss at 38 °C in the presence of cyanide (Table 2). Active aerobic metabolism at 38 °C, however, did not lead to restoration of mitochondrial K$^+$ levels, although supporting reaccumulation of total slice K$^+$. Mitochondria accounted for rather less than 10% of the total K$^+$ content of the slices.

We have shown that addition of La^{3+} to the homogenisation medium inhibits the accumulation of both endogenous and exogenous Ca^{2+} by liver mitochondria to an extent sufficient to permit determination of the quantities of intra- and extramitochondrial Ca^{2+} that existed in the intact cell. The results of Table 2 provide direct experimental evidence that the Ca^{2+}-accumulating system of mitochondria functions in the intact liver cell and that the accumulation is reversible, thus complementing the findings of Rose and Loewenstein[15] who used intracellular aequorin as an indicator of cytosolic Ca^{2+} in intact cells of a salivary gland. Accessibility of Ca^{2+} to, and its retention by, the mitochondria seems to be controlled to a large extent by an opposing energy-dependent mechanism which maintains a low total cellular Ca^{2+}. This calcium-extruding system may be situated either in the plasma membrane[7] or in a region of the endoplasmic reticulum communicating with the extracellular fluids[5]. Inhibition of the extruding system at 1 °C permits entry of Ca^{2+} into the cytosol from the exterior, and from there to be accumulated by the mitochondrial system which remains active at 0 °C (ref. 4). Restoration of aerobic metabolism at 38 °C leads to withdrawal of Ca^{2+} from the mitochondria due, presumably, to a greater affinity of the extruding system at the cell surface The fall of mitochondrial Ca^{2+} at 38 °C in the presence of cyanide, in spite of partial inhibition of extrusion from the cell, is probably due to a more complete inhibition of the mitochondrial system (which is totally dependent on respiration) than of the system at the cell membrane, since the latter would be able to utilise ATP derived from the continuing glycolysis. These considerations suggest that mitochondria may be able to regulate Ca^{2+}-dependent aspects of cell metabolism as postulated by a number of workers (for example, refs 1, 2, 16 and 17), but that this regulatory effect would itself be modulated by the activity of the calcium-extruding system at the cell boundaries.

Supported in part by the US Public Health Service.

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Increasing intracellular sodium triggers calcium release from bound pools

An increase in the free ionised cytosolic Ca^{2+} concentration is generally regarded as the critical event necessary for the induction of exocytotic release of neurotransmitters and hormones from excitable cells. Stimulation of such cells is thought to result in an alteration of the membrane permeability to Ca^{2+} with a subsequent flow of Ca^{2+} ions down their gradient into the cytosol from the extracellular space[1]. In addition, release of Ca^{2+} from intracellular compartments —notably the mitochondria—mediated by a rise in intracellular cyclic AMP may also, in some systems, result in an increase in the cytosolic Ca^{2+} concentration and thus to exocytotic release[2]. Recent evidence suggests that small increases in Na$^+$ concentration may release Ca^{2+} from mitochondria isolated from cardiac muscle cells[3]. Conversely, the uptake of Ca^{2+} into mitochondria of squid axoplasm is reduced by elevated sodium[4]. Since increases in the internal Na$^+$ concentration occur during stimulation of many excitable cells, it is feasible that Na$^+$ could act as a physiological releaser of Ca^{2+} from intracellular stores. We have obtained evidence for this possibility from studies designed to examine the role of Na$^+$ in the calcium-dependent exocytotic release of insulin from the pancreatic β cell.

Pancreatic islets of Langerhans were isolated from 8-week-old male albino rats (200–250 g) and placed in a perifusion system which permitted the temporal monitoring of insulin release from their β cells[5]. Using this system, the effects of veratridine, an alkaloid well known for its ability to increase Na$^+$ permeability in nerve cells[6], were examined to test the possible role of Na$^+$ in Ca^{2+}-dependent insulin release. Our studies show that veratridine leads to a large release of insulin which is totally blocked by tetrodotoxin (TTX), a specific inhibitor of sodium channels in excitable membranes.

A typical experiment is presented in Fig. 1. Veratridine also stimulates release of neurotransmitters from synaptosomes[7] and noradrenaline from the hypogastric nerve[8],

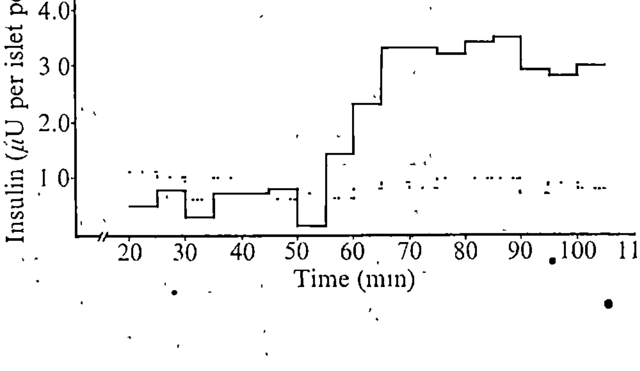

Fig. 1 Islets of Langerhans were isolated by the collagenase technique[13]. An exact number of islets (80–100) were placed in each of two perifusion chambers and perifused with Krebs-Ringer-bicarbonate (KRB) of the following ionic composition: Na$^+$ 139 mM; K$^+$ 5 mM; Ca^{2+} 2.5 mM; Mg^{2+} 1 mM; Cl$^-$ 127 mM; HCO$_3$$^-$ 24 mM. This was supplemented with bovine serum albumin (5 g l^{-1}) and 5.6 mM D-glucose, and maintained at 37 °C and *p*H 7.40 by bubbling constantly with 95% O$_2$ and 5% CO$_2$. Islets in both chambers were exposed to this solution for 45 min, after which one chamber was perfused with KRB containing 100 μM veratridine (*a*) and the other with KRB containing 100 μM veratridine and 3 μM TTX (*b*). The perifusate was collected at timed intervals and the insulin content determined by radioimmunoassay[14] Veratridine caused a fourfold increase in insulin release that was completely inhibited by TTX.

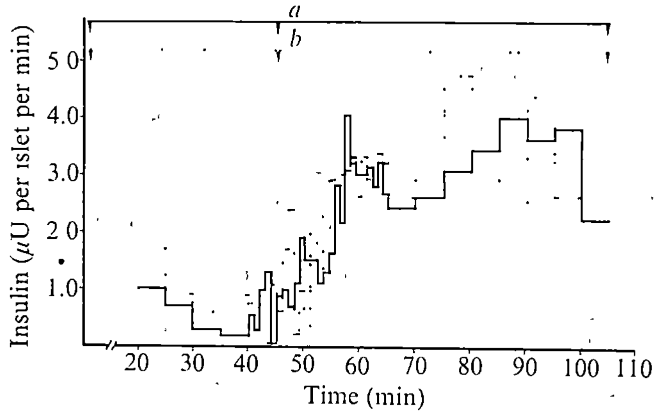

Fig. 2 Same procedure as in Fig. 1. After being perifused for 45 min with KRB alone, islets in one chamber were perifused with KRB containing 100 μM veratridine (*a*) and the others with Ca^{2+}-free KRB supplemented with 100 μM veratridine and 1 mM EGTA to chelate any remaining extracellular Ca^{2+} (*b*). Veratridine caused a fourfold increase in insulin release that was independent of extracellular Ca^{2+}.

release in both systems being blocked with TTX. Our original interpretation was that veratridine increased the intracellular Na^+ concentration by the maintained opening of Na^+ channels in the β-cell membrane, thus depolarising the membrane and opening voltage-sensitive Ca^{2+} channels Ca^{2+} may, in addition, have entered the cell by inward transport in exchange for outwardly transported Na^+, by counter-ion transport mechanisms similar to those identified in the squid axon[8].

Repetition of our experiments, however, in Ca^{2+}-free buffer, containing 1 mM EGTA to chelate any remaining extracellular Ca^{2+}, resulted in the same amount of veratridine-stimulated insulin release as when Ca^{2+} was present (Fig. 2). Since insulin release is always thought of as being strictly Ca^{2+} dependent[10], we suggest that increasing intracellular Na^+ concentration causes release of Ca^{2+} from intracellular compartments. Another method of increasing the intracellular Na^+ concentration is to poison the Na^+ pump with ouabain[11]. We have demonstrated that ouabain also causes insulin release in the absence of extracellular Ca^{2+}. Ouabain-induced release of acetylcholine from neuromuscular endplates also occurs in the absence of Ca^{2+} (ref. 12). Thus insulin release in the absence of Ca^{2+} is not a phenomenon specific to veratridine, but one related to the elevation of intracellular Na^+. In the light of these findings consideration must be given to the possibility that Na^+, by releasing Ca^{2+} from intracellular pools, is acting as a 'second messenger' in exocytotic release processes.

We thank Miss E. Stöcklin for assistance.

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Received December 8, 1975; accepted January 29, 1976

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Interganglionic variation in cell body location of snail neurones does not affect synaptic connections or central axonal projections

RECENT accounts of invertebrate nervous systems have emphasised constancy in the occurrence and electrical properties of large identifiable neurones[1–5]. That constancy, however, has limits, and some giant neurones show variation in peripheral axonal projections[1] and dendritic morphology[6–8]. Smaller neurones increase in number during post-embryonic growth in several groups of invertebrates[9]. During electrophysiological mapping experiments on the brain of the pulmonate pond snail, *Lymnaea stagnalis* (L.), we have found two giant neurones whose positions varied considerably between ganglia, although the variation in cell body position was not associated with any variations in the synaptic connections of the neurones with each other or in identifiable synaptic inputs from other neurones, or with variations in the final projection areas of the principal axons within the brain. Some variation in the detail of axon branching was found to be correlated with cell body location for one peripheral projection.

Intracellular recordings were made from the cell bodies of neurones in the isolated brain maintained in snail blood, using standard electrophysiological techniques. Glass microelectrodes were filled with 2 M K_2SO_4 or 6% Procion Yellow M-4RS. Procion dye was injected intracellularly by iontophoresis[10] to determine axonal morphology of one or two cells in a particular brain Brains were processed histologically in the conventional way. Axonal morphology was reconstructed from superimposed camera lucida drawings of serial sections. No detailed analysis of dendritic morphology was made.

The two neurones which show interganglionic variation in cell body location were called LP2 and LP3 and could not be separated from one another on the basis of their electrical and morphological characteristics. Their bright orange cell bodies were 100–150 μm in diameter depending on snail weight, and the combination of size, positions, electrical properties and morphology of LP2 and LP3 made them uniquely identifiable in the brain of *Lymnaea.*

The somata of LP2, LP3 occurred as a pair in the visceral ganglion (49 snails) or left parietal ganglion (38 snails) or one in each of these ganglia (23 snails). Less frequently they occurred as a pair in the left pleural ganglion (3 snails) or one in the left pleural ganglion and one in the left parietal ganglion (2 snails) or one in the left pleural ganglion and one in the visceral ganglion (1 snail). The location of cell bodies within these three ganglia was limited to small areas of the ventral surface with typical positions shown in Fig 2.

The alternative positions of LP2, LP3 cell bodies did not affect the connections between the two cells or connections with other interneurones. Resting membrane potentials were within the range −45 to −60 mV, and the cells were invariably connected by an electrotonic synapse, even when the cell bodies were in different ganglia (Fig 1*a* and *b*). The junction between the cells was non-rectifying, and the coupling ratio for square pulses was in the range 0.05–0.15.

Spontaneous chemically mediated inhibitory postsynaptic potentials (i.p.s.p.s) were recorded in LP2 and LP3 originating from unidentified interneurones within the brain (Fig. 1, *c–e*). i.p.s p.s were found to be remarkably synchronous and often of similar amplitude in pairs of LP2 and LP3 (Fig. 1 *c–e*). This shared input was seen wherever the cell somata were located. In many preparations two identifiable unitary i p s.p.s were seen (Fig. 1 *c–e*, i.p.s.p.s 1 and 2).

Procion dye injections of LP2 and LP3 showed that they were monopolar cells with two principal axonal routes through the brain. The largest axon projected from the cell body anteriorly and then ventrally to the left pedal ganglion ("pedal axon", Figs 1*f* and 2) while a second finer branch, originating from the larger close to the cell body, projected posteriorly to the visceral ganglion ("visceral axon", Figs 1*f* and 2). When both neurones occurred in the same ganglion their axonal pathways through the central neuropile were extremely similar (Fig. 1*f*), although there were sometimes differences in the peripheral nerves into which they projected. When the cell bodies were in different ganglia their proximal axons met in the brain neuropile and then shared common pathways to the same final ganglion. To reach the same destination irrespective of cell body location required different lengths of pedal and visceral axons (Fig. 2).

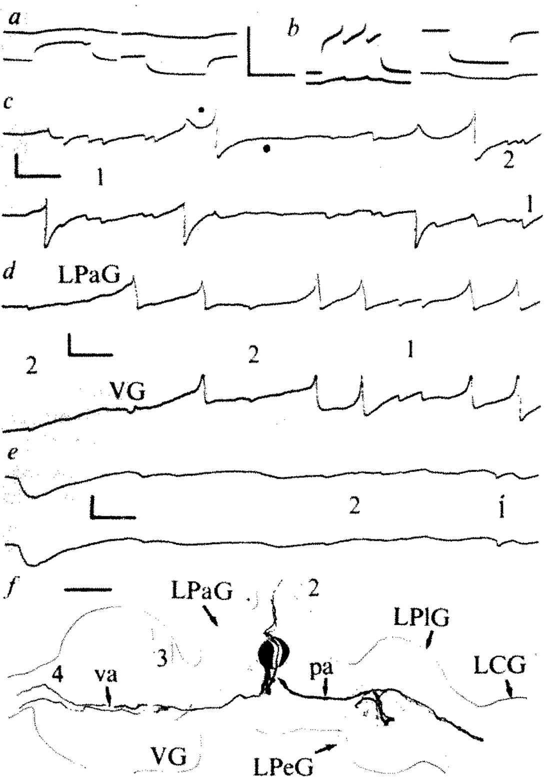

Fig. 1 Electrical properties and morphology of identified *Lymnaea* neurones, LP2 and LP3. *a*, Non-rectifying electrotonic synapse between a pair of neurones whose cell bodies were in the visceral ganglion. Two electrodes were placed in one cell body (bottom traces), one used for recording and the other for current injection, and a third electrode in the second cell body to record the postsynaptic response (top traces). 1-s square current pulse, 2×10^{-9} A, both polarities. Coupling ratio is 0.12 for depolarising (left-hand trace) and hyperpolarising currents (right-hand trace). *b*, Same but for neurones with cell bodies in the visceral ganglion (top traces) and left parietal ganglion (bottom traces). 1×10^{-9} A square current pulse, 1-s duration injected into the visceral neurone by the recording electrode. Coupling ratio for left and right traces, 0.07. Scale bars refer to *a* and *b*. *a*, horizontal, 0.5 s; vertical, 20 mV (top traces), 40 mV (bottom traces). *b*, Horizontal, 0.5 s; vertical, 80 mV (top traces), 40 mV (bottom traces). *c–e*, Spontaneous i.p.s.p.s recorded in paired recordings when the cell bodies were located both in the visceral ganglion (*c*) one in the left parietal ganglion (LPaG) one in the visceral ganglion (VG) (*d*) and both in the left parietal ganglion (*e*). Synaptic inputs to pairs of cells are synchronous and of apapproximately equal amplitude. Identified unitary i.p.s.p.s are marked 1 and 2 in records (*c–e*). In *d* continuous small i.p.s.p.s of a third type are present throughout the records and they probably form the principal component of the compound i.p.s.p.s seen at the beginning of the trace in (*e*). *f*, Reconstruction of a pair of Procion-injected neurones with cell bodies on the ventral surface of left parietal ganglion (LPaG). In the animal the left cerebral ganglion (LCG, the most anterior ganglion in the snail) is in the same horizontal plane as the left pleural ganglion (LPlG) the left parietal ganglion and the visceral ganglion (VG) whereas the left pedal ganglion (LPeG) is ventral to the others (dashed outline). Note the similar axonal geometry of the two cells. The visceral axon (va) projects to the anal nerve (4) but not to the cutaneous pallial nerve (3) in both cells. The pedal axons (pa) could not be traced to the pedal nerves although they penetrate the pedal ganglion neuropile. The left parietal nerve branch (2) arises from the visceral axon in one neurone (shaded) and the pedal axon in the other (unshaded). Scale bar, 200 μm.

One peripheral nerve axon branch of LP2, LP3 could originate from either principal axon depending on the ganglion in which the cell body was located. This was the constantly occurring left perietal nerve branch which emerged from the principal axon at a point where it passed close to the left perietal nerve root (in 37 out of 38 injected neurones). When the cell body was in the left pleural ganglion (3 cells injected) the branch originated from the visceral axon (Fig. 2*a*) but when the cell body was in the visceral ganglion (11 cells injected) it branched from the pedal axon (Fig. 2*c*). In cell bodies located in the left parietal ganglion it mostly branched from the pedal axon (in 21 injected cells) but occasionally from the visceral axon (in 3 injected cells) (Figs 1*f* and 2*b*). Thus the axon branch to the left perietal nerve is formed during development from whichever principal axon passes through the central neuropile of the left parietal ganglion.

Some axon branches to peripheral nerves were variable

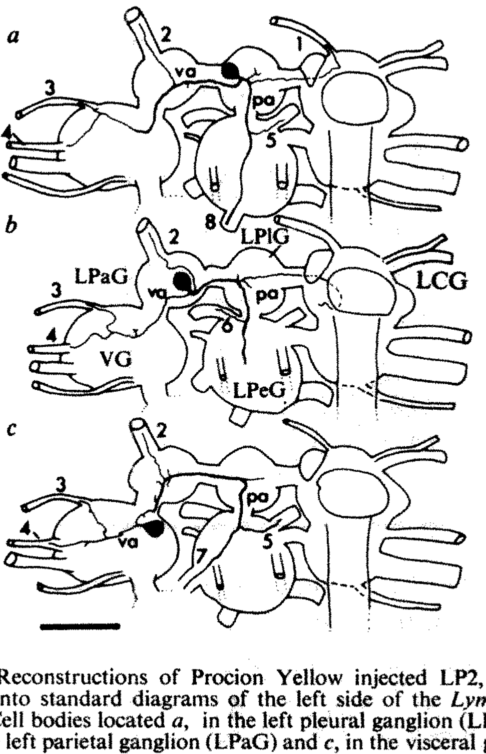

Fig. 2 Reconstructions of Procion Yellow injected LP2, LP3 drawn into standard diagrams of the left side of the *Lymnaea* brain. Cell bodies located *a*, in the left pleural ganglion (LPlG); *b*, in the left parietal ganglion (LPaG) and *c*, in the visceral ganglion (VG) on the ventral surfaces are shown for comparison. For simplicity only one of the LP2, LP3 neurones is included in each diagram. If the second cell occurred in the same ganglion as the one shown in *a–c* then its cell body was located next to the first (see Fig. 1*f*) and its central axonal projections were similar. The left cerebral ganglion (LCG) is most anterior in the brain and the left pedal ganglion (LPeG) most ventral. The cell body location determines the lengths of the visceral axon (va) and the pedal axon (pa). In the sequence *a–c* the visceral axon gets shorter and the pedal axon longer. The left parietal nerve branch (2) originates from the visceral axon in (*a*) and the pedal axon in (*b*) and (*c*). All three cells possess a peripheral axonal branch to the anal nerve (3) and the cutaneous pallial nerve (4). The axon branches to the pedal nerves are variable (see text for discussion). Only in the case of the cell located in the left pleural ganglion (*a*) was it possible to trace the cerebral ganglion axon branch to the nuchal nerve (1). Labelling of pedal ganglion nerve roots; 5, superior cervical nerve; 6, inferior cervical nerve; 7, inferior pedal nerve; 8, medial pedal nerve. Scale bar, 500 μm.

in occurrence. The apparent variability of pedal nerve projections seen in Fig. 2 may partly be due to problems of tracing distal nerve processes which have little dye in them, but in the visceral ganglion (Figs 1*f* and 2) axon branches were easy to map and some variation in branching pattern is certain. This was not obviously related to the location of the cell body. Thus in some snails the visceral axon projected to the anal nerve (this occurred in four cell bodies located in the visceral ganglion, and four cell bodies located in the left parietal ganglion), in others it projected to the cutaneous pallial nerve (in three cell bodies located in the visceral ganglion, one cell body located in the left parietal ganglion and one cell body located in the left pleural ganglion) or it projected to both these nerves (in three cell bodies located in the visceral ganglion and in three cell bodies located in the left parietal ganglion).

The constant presence of certain synaptic connections which LP2 and LP3 make shows that the position of cell bodies need not be constant for normal neural connections to be made. It seems more important to specify axonal projections rather than cell body position and this is suggested by the present study as well as that of Selverston and Mulloney[11] where cells from the lobster stomatogastric ganglia varied considerably in cell body location but had constant neuropile axon structure.

I thank Carole Slade for help with the anatomical part of the work, Professor G. A. Kerkut, Drs M. Burrows, T. S. Collett, D. A. Dorsett, M. F. Land, R. M. Pitman, N. V. Swindale and W. Winlow for their comments on drafts of this manuscript and the MRC for financial support.

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Received November 4, 1975; accepted February 3, 1976.

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Absence of a plateau in length–tension relationship of rabbit papillary muscle when internal shortening is prevented

IN the length–tension relationship of the isolated papillary muscle there is a region in which increased muscle length is associated with an increased force of contraction. Several problems make it difficult to determine the physiological basis for this relationship. Thick and thin filament lengths and sarcomere lengths have not been determined with the same accuracy in heart muscle as in skeletal, though there is evidence that these lengths are nearly the same in both muscles[1–3]. Papillary muscle cannot normally be tetanised or made to contract at low temperatures, and there is reason to believe that it is not fully activated by a single stimulus in the usual bathing medium[4,5]. In at least some papillary muscles there is considerable internal shortening during a twitch contraction[6,7]. Such length changes during contraction could change the amount of force developed by affecting the amount of thick and thin filament overlap and by producing shortening deactivation[8,9]. We have developed a photoelectronic servo apparatus similar to that developed by Gordon *et al.* (see refs 10 and 11 for details of operation) for use with papillary muscles to hold constant the length of a segment of preparation during contraction.

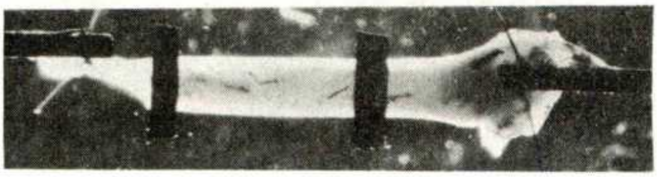

Fig. 1 A low power view of a typical rabbit papillary muscle preparation. The tapered tendon end and cut end are knotted to stainless steel wires with 10-0 nylon monofilament sutures. As a reference length, the diameter of the wires was 200 μm. The muscle was stretched to L_m. Its diameter in the uniform central region was about 500 μm, while its total length (knot-to-knot) was about 3.7 mm. The markers were about 1 mm long by 0.25 mm wide and were made from gold foil (2.5 μm thick), folded over along each long side, so that total marker thickness was 5 μm. Markers were glued to the surface of the papillary muscle with an epoxy adhesive able to cure underwater. The length between the markers (edge-to-edge) was about 1.7 mm. The dark streaks and spots visible in the muscle are collections of opaque 15-μm diameter microspheres; their presence does not influence muscle function.

Small diameter papillary muscles were obtained from the right ventricles of anaesthetised rabbits (about 0.5 kg). When these muscles were stretched to near L_m, the length at which peak developed tension is maximum, the diameter measured in the mid-region was about 500 μm, and striation patterns could be observed and recorded as described before[6]. The bathing medium was a modified Krebs–Ringer solution bubbled with a 95% O_2–5% CO_2 mixture. The solution contained (mM): NaCl, 117; $NaHCO_3$, 1.4; KCl, 4.2; $CaCl_2.2H_2O$, 2.5; $MgSO_4.7H_2O$, 1.2; NaH_2PO_4, 1.2; HEPES buffer, 7.4, and glucose, 5.6.

When a suitable muscle was obtained, it was tied to wires[6] (Fig. 1), which were connected to a servo motor and force transducer. The muscle was stretched slowly to just beyond L_m while being stimulated repeatedly. The experimental chamber was then drained of solution and gold markers (Fig. 1) were rapidly attached (within several minutes) to a central, uniform segment of the papillary muscle. The chamber was then refilled with solution and stimulation resumed. Comparison of length–tension relationships before and after marker attachment revealed no significant differences, and the muscles contracted vigorously for several hours after marker application. Microspheres which had been injected into the beating heart and subsequently lodged in the papillary muscle capillary network, were examined microscopically during fixed-end contractions to ensure that the markers moved synchronously with other elements distributed throughout the muscle cross section. Such synchrony was taken to indicate that during servo control, internal length does not change when the separation between the markers located on the muscle surface is kept constant.

The fixed-end length–tension relationship obtained from a papillary muscle after markers had been attached, but without servo control, is shown in Fig. 2. Peak twitch force declines to nearly zero when total muscle length is decreased by about 25% of L_m, and there is only a very narrow (about 1% of L_m) plateau present over which peak developed force remains near maximal. The steeply rising resting tension made it impossible to stretch the muscle more than a small amount beyond L_m. Servo control was used at each of the lengths indicated by arrows in Fig. 2 to prevent changes in marker separation. In each case, control was increased progressively to the point where only a small change in marker separation occurred during a twitch. Further increases in servo gain were avoided, since at higher gains the servo system became marginally stable. Only the record obtained at the longest indicated length is shown in Fig. 3, the others being similar in form. As the change in marker separation is progressively reduced, there are significant increases in the rate of rise of force and twitch peak amplitude. There is also an increase in the half time for twitch tension decay and a tendency for the twitch peak to occur slightly earlier.

Data obtained from each of the five records of the kind just described are plotted in Fig. 3, where it can be seen that the relative length error decreases monotonically as servo gain is increased. At maximum gain, the relative length error is less than 1%, and is even smaller at the two longest lengths, where large resting tensions were present. The extrapolated values give the best estimates of the force which would be present if marker separation had been held absolutely constant during a twitch contraction. The extrapolated values for the forces do not differ much from the values observed with minimum relative length error. These results provide a reasonable basis for belief that the small residual length error present did not significantly affect the force responses, so that the active tension developed indicates unambiguously force generation by the contractile component.

It is now possible to plot peak developed force against the length between the markers at maximal servo system gain (Fig. 4). The largest relative marker separation corresponds to a muscle length very near to L_m. The average sarcomere length corresponding to the greatest length between the markers was found by photographing the striation pattern[6] in the resting muscle. A mean value of 2.3 μm was obtained for the average sarcomere length at a marker separation corresponding to L_m in three muscles. In Fig. 4 the sarcomere length corresponding to a 15% decrease in marker separation 2.0 μm, was calculated by assuming a linear decrease of resting sarcomere length with marker separation, a good approximation in the sarcomere length range under consideration[6]. Figure 4 shows that there is a marked variation of twitch force within a range of average sarcomere lengths from about 2.0 to 2.3 μm, which contrasts with the results for frog skeletal muscle fibres in which isometric tetanic tension is essentially constant within this range[11].

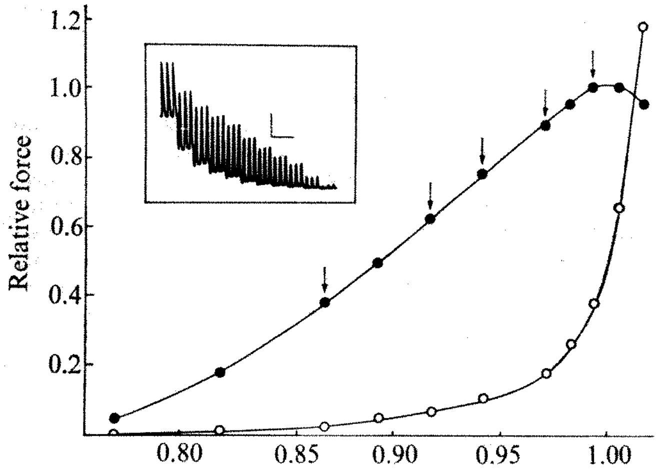

Fig. 2 A plot of peak developed (●) and resting (○) tension against muscle length for a rabbit papillary muscle. Data were obtained from the experimental record shown in the inset. Forces are expressed relative to the amplitude of the maximal peak developed twitches. Total muscle length (knot-to-knot) is expressed relative to L_m. Inset: length–tension relationship of a rabbit right ventricular papillary muscle. Markers had previously been attached as shown on a different muscle in Fig. 1. The muscle was first stretched slowly (not shown) until peak developed twitch tension passed a maximum and then declined slightly. After this, muscle length was decreased progressively allowing three twitches at each length. At the shortest length, resting tension was nearly zero. The amplitude of the twitches after the first and second decreases in length was nearly equal. Total muscle length (knot-to-knot) was 4.1 mm at the greatest length and 3.1 at the shortest length. The size of the length decreases in order were (μm): 50, 50, 50, 50, 100, 100, 100, 100, 200 and 200. Rate of stimulation was 17 per min, temperature was 22 °C. Calibration lines indicate 182 mg (vertical) and 14.4 s (horizontal). At the length corresponding to that after the second length decrease, muscle cross-sectional area was about 0.09 mm^2, based on microscopic determination of diameter in the central region between the markers. Maximal peak developed twitch tension was about 5 g mm^{-2}.

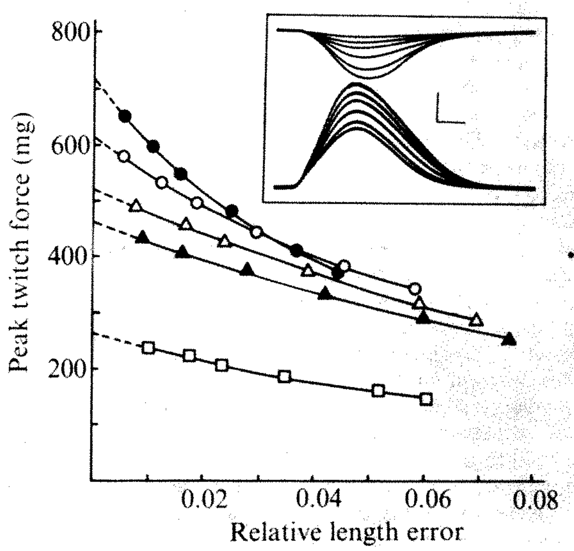

Fig. 3 Peak twitch force plotted against relative length error. Data were obtained at each of the positions indicated by arrows in Fig. 2 from records of the kind shown in the inset. In each case, as servo gain increased progressively, the maximum decrease in length produced by each twitch was obtained with the corresponding twitch amplitude. Decreases in length were expressed relative to the initial, resting length between the markers, so that a relative length error was obtained. Each set of data appears in sequence from top to bottom in correspondence with the arrows in Fig. 2. As servo gain was increased, the amount of relative length error decreased, that is, a nearly perfect isometric clamp was attained. In each case, the points are well fitted by a smooth curve. The extrapolations of these curves (dashed line segments) on to the force axis give good estimates of the true isometric forces obtainable if marker separation were held constant during a twitch. In sequence from top to bottom, the initial, resting marker separations were (mm): 1.60, 1.56, 1.52, 1.48 and 1.37. Inset: a sequence of length and force records showing the effects of increasing amounts of servo control applied to the papillary muscle from which the record shown in Fig. 2 was obtained. The upper series of traces are length signals from the photoelectronic spot follower. These indicate changes in marker separation, where length decreases are indicated by a downward deflection. A corresponding series of twitches is shown below. The smallest twitch is associated with the largest deflection in the length signal records. Between each twitch, the gain of the servo system was increased. This caused the end of the muscle attached to the servo motor to be driven so as to diminish progressively the amount of change in marker separation during a twitch. Finally, servo gain was increased to the point at which, during a twitch, there was a just perceptible deflection in the length signal trace. These records were obtained at the resting total muscle length indicated by the arrow in Fig. 2 closest to the active force peak. Similar sequences were obtained at the other four positions indicated by arrows in Fig. 2, but they are not shown here. At maximum gain, an error of 1 μm in the length between the markers causes an opposing force of about 73 mg at the end of the muscle attached to the servo motor. The vertical calibrating line indicates 44 μm or 182 mg, and the horizontal calibrating line indicates 200 ms. The largest twitch amplitude corresponds to a tension of about 8 g mm^{-2}.

The results presented here indicate that most of the ascending limb of the length–tension relationship of rabbit

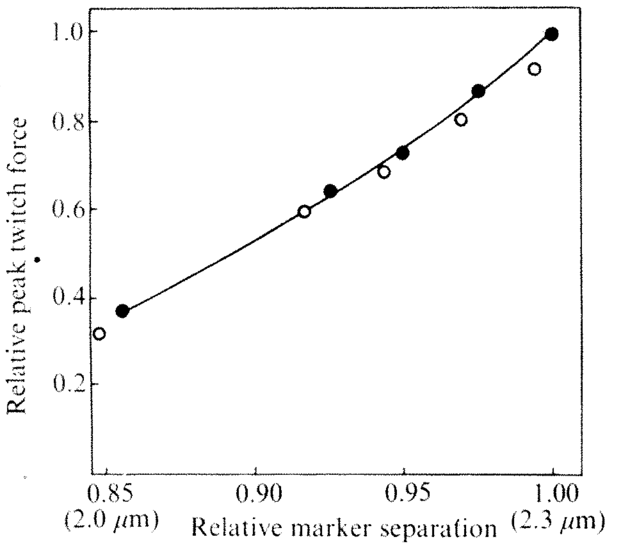

Fig. 4 Peak twitch force as a function of marker separation. Data were obtained from the graph shown in Fig. 3. All forces are expressed relative to the largest obtained by extrapolation, and all marker separations are expressed relative to the longest initial, resting value, 1.60 mm. ●, Plot of extrapolated force values against marker separation for the case of zero relative length error, that is the best estimates for perfectly isometric conditions. ○, Actual final values for forces and marker separations under maximum servo gain conditions. Note that a decrease in marker separation of only about 15% leads to a decrease in force of more than 60%. Average sarcomere lengths are given in parentheses. The value 2.3 μm was obtained by microscopic examination of the resting muscle at marker separation 1.60 mm. The value 2.0 μm was obtained by assuming a linear decrease of resting sarcomere length with marker separation.

papillary muscle cannot simply be explained on the basis of variation in the amount of thick and thin filament overlap, and, therefore, numbers of cross bridges available for interaction, or by changes in the amount of filament overlap and deactivation caused by internal shortening during contraction. Results obtained from skeletal muscle suggest that a variation in level of activation with muscle or sarcomere length is a significant factor[12-14]. This is, of course, not a new proposal[15-18]. Recent experiments with skinned muscle cells obtained from rat ventricles are interesting in this regard[17]. If these cells, which have had their sarcolemmae removed mechanically, are activated directly by high concentrations of calcium ion in the presence of a large Ca^{2+}-buffering capacity, the resulting sarcomere length–tension relationship resembles that obtained from living frog skeletal fibres[11]. Steady tension varies by only about 10% in the range of sarcomere lengths from about 1.7 to 2.3 μm, and it varies even less within the range 2.0 to 2.3 μm. But when the skinned cells were made to contract phasically by inducing a Ca^{2+}-triggered transient release of Ca^{2+} from the sarcoplasmic reticulum (SR), tension decreased substantially as sarcomere length was reduced below 2.3 μm. In the work with living papillary muscles reported here, tension decreased even more rapidly as sarcomere length was reduced below 2.3 μm than in the phasic contractions of skinned heart cells[17]. This suggests that in the living muscle the mechanism responsible for regulating the amount of activating calcium released in a twitch is not necessarily the same as that which governs the calcium-triggered release of calcium from the SR of skinned cells.

This work was supported by grants from the National Institutes of Health, the US National Science Foundation, the Muscular Dystrophy Associations of America, the American Heart Association and the Massachusetts Heart Association. R.L.M. was a National Institutes of Health postdoctoral fellow.

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Received January 12; accepted January 28, 1976.

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Electrophysiological measurements of spectral sensitivity of central visual cells in eye of blowfly

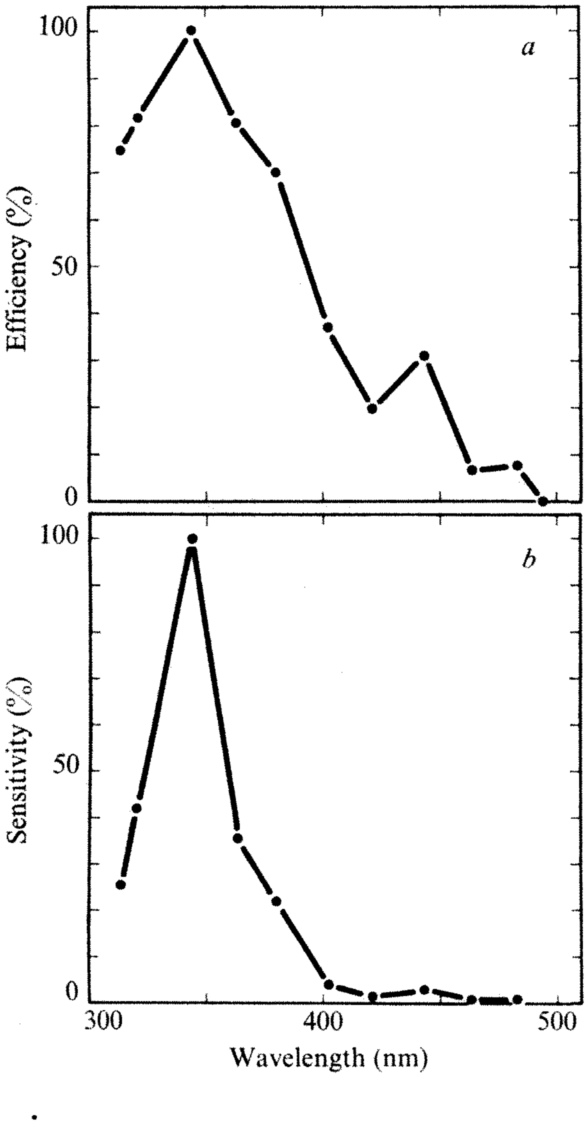
IN 1913 von Frisch was able to show that bees can discriminate between different colours. He thus refuted the then prevailing opinion that only certain vertebrates have colour vision. Since then insect colour vision has become a significant subject of research, the more so in view of the fact that using insect visual cells the transformation of light into neural excitation can be readily examined. In recent years numerous electrophysiological, microspectrometric and behavioural experiments have been carried out to determine the spectral sensitivity of visual cells not only of the bee but also of the fly. In the case of the blowfly *Calliphora erythrocephala*, each compound eye is made up of approximately 3,000 ommatidia. Under the lens of each ommatidium there are eight visual cells, and these can be divided into two groups according to their shape. Six of them (numbered R$_{1-6}$) have short axons which end in the first optic ganglion (lamina ganglionaris). The remaining two visual cells (R$_{7+8}$, also called 'central visual cells' because of the position they occupy in the ommatidium) are characterised by their long axons, which stretch from the retina through the lamina ganglionaris down to the second optic ganglion (medulla).

Measurements by electrophysiological methods have given rise to double-peaked sensitivity curves[1-3], all having in common a maximum in the ultraviolet range at 350 nm. The other peaks occurred at different wavelengths in the visible range of the spectrum. According to the positions of these maxima Burkhardt[1] was able to divide the sensitivity curves into three categories which he ascribed to three colour receptors (blue receptor: λ_{max} = 475 nm, green receptor: λ_{max} = 493 nm, yellow-green receptor: λ_{max} = 515 nm). The frequency of occurrence found for the sensitivity curves in each category led him to ascribe the green receptor to visual cells R$_{1-6}$; and the blue and yellow-green receptor to central visual cells R$_{7+8}$. Microspectrometric measurements[4] confirmed this assumption in the case of visual cells R$_{1-6}$. For the visual cells R$_{7+8}$ together (these are positioned in line with one another and share one rhabdomere) absorption spectra were found which, in addition to an ultraviolet maximum, had a maximum at 475 nm. Electrophysiological measurements with simultaneous histological localisation of the cells in question showed that the spectral sensitivity with a maximum at 490 nm did in fact come from the visual cells R_{1-6}. Such a definite proof has not been possible for visual cells R_{7+8} taken separately.

To determine the spectral sensitivity of the central visual cells R_{7+8}, intracellular potentials were recorded from the first optic chiasma. Since the axons of the visual cells R_{1-6} already terminate in the lamina we could be sure that whenever depolarising potentials with shapes typical for generator potentials from a retinula cell occurred, the recordings from the chiasma came either from the seventh or eighth visual cell. The measurements were carried out on the dark-adapted eye of male blowflies *Calliphora erythrocephala* Meig. with normal eye pigmentation. Many attempts were made but only in two cases were the recordings from the central visual cells stable enough to enable complete measurement of the intensity–response curve and of the spectral efficiency. Using intracellular injection of the fluorescent dye Procion Yellow and subsequent histological examination, the exact recording site in the chiasma could be located. In one case only a part of a central visual cell became stained and therefore it was not possible to distinguish exactly between R_7 and R_8.

Figure 1 shows the spectral efficiency curve of a central visual cell (response elicited by light stimuli with the same quantum level at all wavelengths) and its spectral sensitivity curve (reciprocal of quantum level eliciting the same response at all wavelengths). The spectral efficiency (Fig. 1*a*) of this cell has its maximum in the ultraviolet range at 344 nm, and drops sharply as the wavelength increases. At 443 nm it shows a small subsidiary shoulder and at 500 nm the spectral efficiency is almost zero. The spectral sensitivity curve (Fig. 1*b*) shows very clearly a single-peaked ultraviolet receptor. Here, the subsidiary shoulder in the blue range has declined to a level 10% below maximum sensitivity. Figure 2 shows the spectral efficiency and sensitivity curve of another central visual cell, differing in shape from that in Fig. 1. The efficiency curve (Fig. 2*a*) also shows a maximum in the ultraviolet range at 344 nm; but in this case the other maximum at 443 nm in the blue range was much more evident than in Fig. 1. At 500 nm the efficiency of this receptor is considerable and is still evident above 550 nm. The sensitivity curve (Fig. 2*a*) also shows two maxima the positions of which correspond with those in Fig. 1. The height of the blue maximum, however, is different. The axon of the cell mentioned in Fig. 2 was stained in the region between medulla and basement membrane. Faint staining could also be seen in the proximal region of the retina. Here, it was impossible to ascertain whether a seventh or eighth visual cell had been stained.

Fig. 1 Spectral efficiency (*a*) and spectral sensitivity (*b*) of a central visual cell from eye of *Calliphora*. Single-peaked ultraviolet receptors of this kind were previously unknown in the blowfly eye.



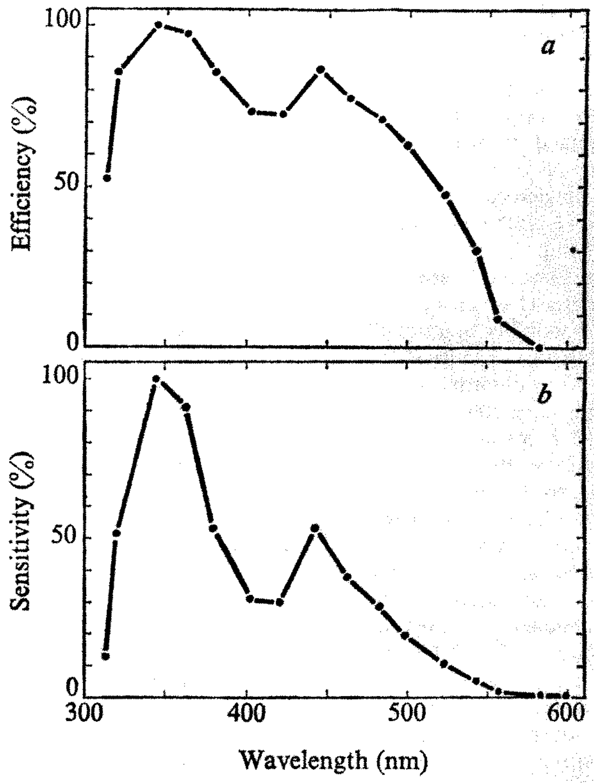


Fig. 2 Spectral efficiency (*a*) and spectral sensitivity (*b*) of another central visual cell which in addition to the ultraviolet maximum also shows a marked maximum at 443 nm in the blue range.

In electrophysiological experiments on the spectral sensitivity of visual cells in the eye of *Calliphora*, only double-peaked sensitivity curves have so far been found. Thus the occurrence of a single-peaked ultraviolet receptor is at first surprising[5]. The spectral sensitivity curve (Fig. 1*b*) suggests the existence of a special ultraviolet pigment in the rhabdomere of the seventh or eighth visual cell, such as has been isolated in *Ascalaphus*[6]. Ultraviolet receptors have also been found in other dipteran compound eyes[7-9]: thus they seem to be a characteristic of a number of dipterans. Also, the double-peaked sensitivity curves of the visual cells R_{1-6} in *Calliphora* can be ascribed to two separate pigments[3]. It is thus evident that the curves shown in Fig. 2 result from two visual pigments with maxima at 344 nm (ultraviolet pigment) and at 443 nm (blue pigment). The occurrence of such a blue pigment does not contradict earlier microspectrometric measurements on the rhabdomere of visual cells

R_{7+8}, for it is now well known that a stable metarhodopsin forms during these measurements, which shifts the maximum of the absorption curve towards longer wavelengths (H. Langer, personal communication).

We could not be certain which spectral sensitivity curve belonged to which visual cell; R_7 or R_8. Experiments on *Drosophila*[8] have shown, however, that the seventh visual cell is a single-peaked ultraviolet receptor whereas the eighth visual cell is a double-peaked ultraviolet–blue receptor. If one assumes that this also holds good for *Calliphora* the ultraviolet receptor (Fig. 1) would be the seventh visual cell and the ultraviolet–blue receptor (Fig. 2) the eighth visual cell. There remains, however, the possibility that not all the seventh visual cells and not all the eighth visual cells show uniform spectral sensitivities. Thus, the two different spectral sensitivity curves which we obtained might have come from two cells of the same type. If the ultraviolet pigment–blue pigment ratio varies from cell to cell this would be one explanation for the different maxima observed. This, for example, was the explanation suggested for the variation in height of maxima of spectral sensitivity curves with two or more peaks in *Eristalis*[9]. Microspectrometric measurements carried out on visual cells R_{7+8} of several dipterans do in fact show that neither of the rhabdomeres 7 and 8 constitutes an homogeneous population of receptors (K. Kirschfeld, personal communication). The question of which curve belongs to which cell type still remains to be answered, but our measurements show that, in addition to receptors with sensitivities found previously, there exist receptors with single-peaked ultraviolet sensitivities and with double-peaked ultraviolet–blue sensitivities. If one leaves out the possible shifting of the position of the blue maximum due to light-guide characteristics of the central rhabdomeres, one can conclude that the vision of the fly is based on at least three photopigments with maxima in the ultraviolet range (about 344 nm), blue range (about 443 nm) and green range (about 490 nm).

Supported by the Deutsche Forschungsgemeinschaft.

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Received November 14; accepted December 31, 1975.

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Membrane noise in *Paramecium*

THE ciliated protozoan *Paramecium* provides an opportunity to study the electrophysiological properties of an excitable membrane in relation with simple locomotor behaviour. The *Paramecium* surface membrane controls the locomotor activity, in particular ciliary reversal (resulting in backward swimming), by ionic conductance mechanisms like those in other excitable cells[1,2]. Depolarising stimuli (electrical, chemical or mechanical) cause a graded receptor potential due to a regenerative transient increase in calcium conductance and a delayed increase of potassium conductance. The resulting Ca^{2+} influx causes an increase in the intracellular Ca^{2+} concentration which initiates the reversal of the ciliary beating direction. Normal beating is resumed when the excess internal Ca^{2+} has been removed by metabolism-dependent processes.

The correlation between depolarisation and the orientation of the cilia was first observed in relation with spontaneous fluctuations of the resting membrane potential[3,4]. Spontaneous ciliary reversals seemed to coincide with voltage fluctuations in depolarising direction.

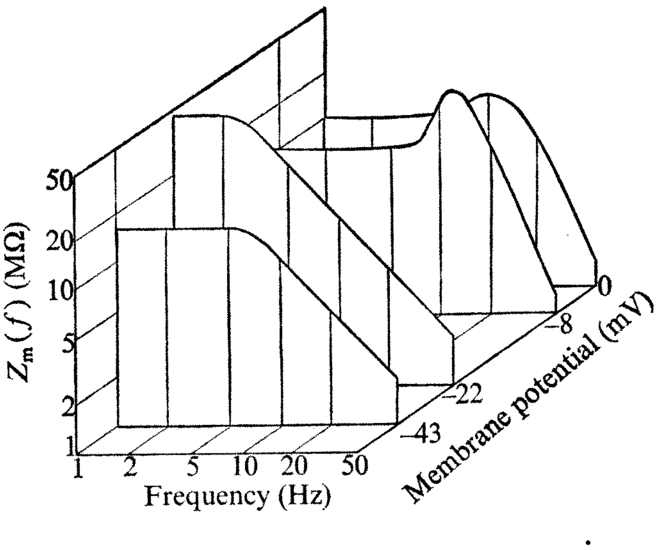
This report deals with the search for the physical origin of these fluctuations ("membrane noise") and with their relationship to spontaneous reversals. Membrane noise voltage (at constant current), although more easily measurable and less contaminated with electronic noise, may give less information about the underlying processes than noise current (at constant voltage), since the noise voltage is filtered by the frequency-dependent membrane impedance. The noise voltage is, however, expected to induce spontaneous ciliary reversals. We therefore measured both noise voltage and noise current and the impedance. We show that the membrane noise belongs to the so called $1/f$ type and that its intensity is related to the magnitude of the passive K^+ flux across the membrane. The noise voltage seems to be filtered by a resonance-like membrane impedance and, finally, we present evidence that $1/f$ noise can occasionally lead to spontaneous ciliary reversals.

Current clamp methods (using two intracellular microelectrodes) similar to those of Naitoh and Eckert[5] were used to record noise voltage from specimens of *Paramecium caudatum*. The noise voltage was perfectly correlated in both electrodes and independent of electrode positions, as expected from the isopotential nature of the *Paramecium* membrane[6]. Noise current was measured with standard voltage clamp techniques. The noise was tested for constancy and processed on an HP 3721 A correlator and Fourier transformed to obtain the spectral density function. The membrane impedance was obtained by cross correlation of injected white-noise current and membrane noise voltage response. In the "standard" bathing solution (4 mM KCl, 1 mM $CaCl_2$, 1 mM Tris, *p*H 7.2) the membrane noise was stationary and no spontaneous reversals were observed.

Figure 1 shows the membrane impedance modulus $|Z_m(f)|$ at different mean membrane potentials, measured from a specimen in the standard solution at 19 °C. At the resting potential (−22 mV) and on hyperpolarisation the membrane behaves as a simple RC circuit. On depolarisation, however, a resonance peak shows up at about 12 Hz. This resonance, which tends to diminish on stronger depolarisation, is reminiscent of the squid axon membrane impedance[7] and accounts for the oscillatory character of the voltage response on a steady depolarising current stimulus[5].

Figure 2*a* shows typical noise voltage spectra, $S_V(f)$, in which

Fig. 1 *Paramecium* membrane impedance modulus $|Z_m(f)|$ at various membrane potentials determined as (cross)power spectra of membrane voltage response to white noise current stimulus. R.m.s. value of injected noise was 5×10^{-11} A. In the standard bathing solution resting potential $V_r = -22$ mV and K^+-equilibrium potential $E_K = -38$ mV at 19 °C.



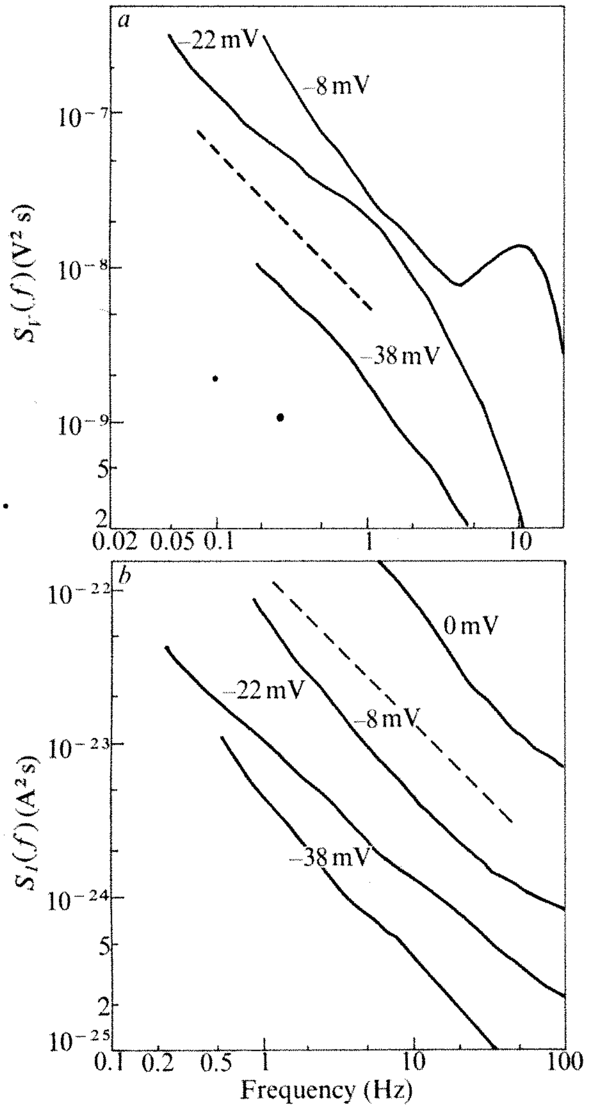


Fig. 2 Power spectra of stationary membrane noise at various membrane potentials. Standard solution at 19 °C. *a*, Noise voltage spectra, $S_V(f)$, measured under current clamp. Electronic noise negligible (10^{-11} V² s at 1 Hz). *b*, Noise current spectra $S_I(f)$, measured under voltage clamp. Electronic noise negligible up to about 50 Hz (10^{-25} A² s), but exceeding membrane noise above 100 Hz. Note the difference in frequency scale. Dashed lines represent slope −1. Spectra are related according to $S_V(f) = S_I(f)|Z_m(f)|^2$.

the impedance filtering effect is clearly reflected. No significant changes in membrane noise spectra nor in other electrical parameters were observed when ciliary beat was inhibited by adding 1 mM $NiCl_2$ to the standard solution. This is consistent with the known independence of ciliary beat on electrical events[2].

Noise current spectra, $S_I(f)$, are shown in Fig. 2*b*. The experimental data satisfy the theoretical conversion formula[8]

$$S_V(f) = S_I(f)\ |Z_m(f)|^2$$

Noise current spectral densities decrease with increasing frequencies. On a log–log plot their shapes suggest a power law fit of the form

$$S_I(f) = Nf^{-\alpha}$$

where N is voltage dependent. α has a value close to 1.0 at rest and tends to increase on depolarisation. Values exceeding 1.5 were not found. This result places the membrane fluctuations of *Paramecium* in the $1/f$-noise category.

This type of noise is also present in nerve fibres[9] and in cultured neuroblastoma cells (W. H. Moolenaar, unpublished), but unfortunately the physical basis of $1/f$ noise is not yet clear[10]. An empirical understanding of *Paramecium* membrane noise can, however, be obtained. Figure 3 shows the dependence of the noise intensity, N, on membrane potential V_m. A minimum value of N was found for V_m between −35 mV and −45 mV, that is the vicinity of the K⁺ equilibrium potential E_K ($E_K = -38$ mV in the standard solution[11]).

Replacement of the standard solution by a high-potassium solution (18 mM KCl, 1 mM $CaCl_2$, 1 mM Tris, *p*H 7.2) roughly eliminates the potassium concentration gradient across the membrane, thereby reducing both E_K and V_m to about 0 mV. In this case membrane noise was no longer measurable since it vanished in electronic background noise, but hyper- or depolarisation raised the membrane noise intensity substantially once again. Although quantitative data on the *I–V* relationship for the separate ionic currents in *Paramecium* have not been reported as yet, the presented data strongly suggest a proportionality between membrane noise intensity and the magnitude of the passive K⁺ flux. We attribute the remaining noise at zero K⁺ flux in the standard solution (at $V_m = E_K$ in Fig. 3) to calcium and nonspecific leakage currents. Metabolism-dependent processes most likely do not contribute to the membrane noise since cooling the specimen to 4 °C did not significantly alter noise spectra.

To relate membrane noise to spontaneous ciliary reversal a low-potassium bathing solution was chosen (1 mM KCl, 1 mM $CaCl_2$, 1 mM Tris, *p*H 7.2). In this solution the noise intensity at resting membrane potential (−25 to −29 mV) was substantially increased, as has to be expected from the mentioned relationship between noise intensity and K⁺ flux, since in this case the steady K⁺ flux is larger. Spontaneous transient ciliary reversals did occur, coinciding with depolarisations ranging from about 3–8 mV in amplitude and about 0.1–1 s in duration. These random depolarisations caused the noise to be non-stationary, which implies that the usual noise processing techniques do not give meaningful results. Under voltage clamp, on the other hand, spontaneous reversals were not observed. In this case noise current was stationary and exhibited a $1/f$ spectrum (intensity $N = 5 \times 10^{-23}$ A² at resting membrane potential).

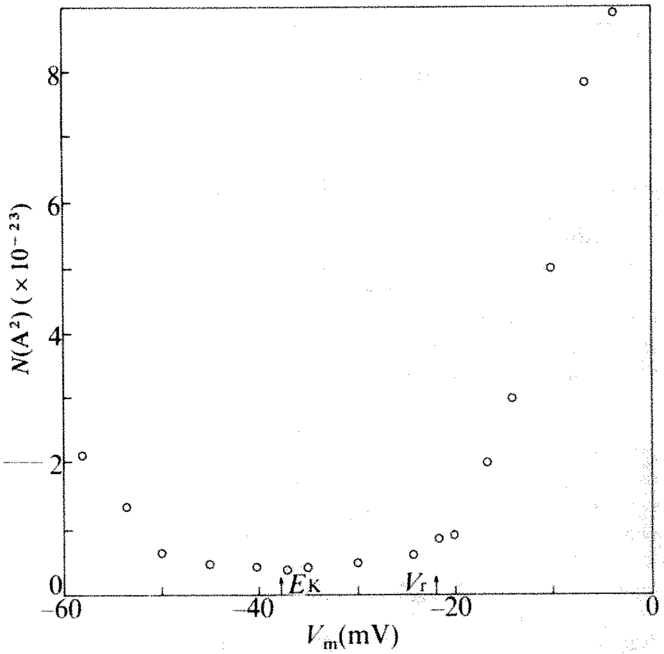


Fig. 3 Intensity N of $1/f$ noise plotted against membrane potential V_m. N was determined as the value of $S_I(f)$ at 1 Hz. Resting potential $V_r = -22$ mV, K⁺-equilibrium potential $E_K = -38$ mV in the standard solution.

We conclude therefore that membrane $1/f$-noise voltage, generated mainly by the steady passive K^+ flux, can activate Ca^{2+} conductance occasionally and thus underlies spontaneous reversals in *Paramecium*.

To our knowledge this is the first report which provides experimental evidence for a connection between membrane $1/f$ noise and simple behavioural effects in a living organism.

This work was supported by a fellowship and grant from the Koningin Wilhemina Fonds and by a grant from the Netherlands Organization for Pure Research (Z.W.O.).

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Antagonistic effects of GABA and benzodiazepines on vestibular and cerebellar neurones

ALTHOUGH benzodiazepines are widely used psychotropic drugs their mode of action within the central nervous system (CNS) is controversial[1–4]. Based on biochemical and pharmacological investigations of various central and peripheral neural substrates, Costa *et al.*[5] and Haefely *et al.*[6] suggested that they act, at least in part, through a facilitation of γ-aminobutyric acid (GABA)-ergic transmission. We have tested this hypothesis electrophysiologically at the single unit level in areas of the CNS in which GABA has been unequivocally shown to be an inhibitory transmitter. Single unit recordings were made in neurones of the lateral vestibular nucleus (Deiters) and in Purkinje cells of the cerebellum.

Deiters neurones receive a monosynaptic GABA-ergic inhibitory input through Purkinje cell axons originating in the cerebellar cortex. There is conclusive evidence for the synaptic release of GABA at the inhibitory afferences of Deiters neurones[7–9]. Furthermore, GABA is released at the level of the inhibitory synapses between the so-called basket cells and the Purkinje cells[10]. Purkinje cells were identified on the basis of the spontaneous "inactivation response"[11] recorded at the appropriate levels of electrode penetration.

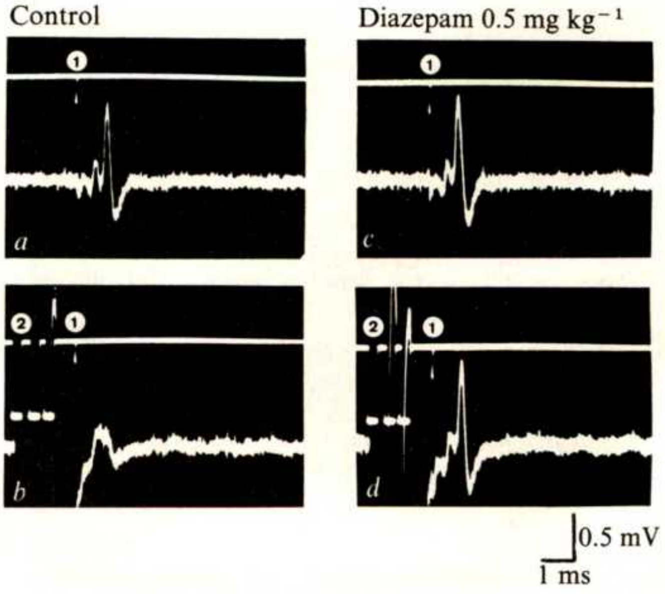
Experiments with Deiters neurones involved three cats anaesthetised with N_2O and artificially ventilated. Deiters neurones were activated by antidromic stimulation of the vestibulo-spinal tract (Fig. 1*a*). The antidromic response was inhibited by the preceding stimulation of Purkinje cell axons (Fig. 1*b*). Intravenous diazepam had no effect on the antidromically evoked action potential (Fig. 1*c*), but readily and reproducibly suppressed monosynaptic inhibition of Deiters neurones evoked by stimulation of the cerebellum (Fig. 1*b*). The inhibitory effect of the stimulation of Purkinje fibres (Fig. 1*b*) could be duplicated by the iontophoretic application of the inhibitory neurotransmitter GABA on to Deiters neurones; antidromically evoked action potentials were reduced by GABA in a dose-dependent manner. Likewise, the intravenous injection of diazepam suppressed this inhibition of the antidromically evoked potential by GABA.

The inhibitory effect of Purkinje cell fibres on Deiters neurones, which was abolished by diazepam (Fig. 1*d*) could be restored either by increasing the intensity of stimulation of Purkinje fibres, or by increasing the amount of microiontophoretically delivered GABA. The effect of increased cerebellar stimulation was in turn antagonised by locally applied bicuculline (compare ref. 12).

The experiments on Purkinje cells were carried out on five Füllinsdorf albino rats (200–300 g body weights) anaesthetised with urethane (1.2 g kg^{-1}, intraperitoneally). In all cases, the spontaneous firing of Purkinje cells could be inhibited completely and instantaneously by small amounts of iontophoretically applied GABA (of the order of 2–5 nA). This inhibition was strikingly affected by a preceding injection of the benzodiazepine derivative bromazepam: a large amount of GABA (10 nA) now required several seconds to suppress spontaneous activity (Fig. 2*a*). The same amount of locally applied GABA could be made even less effective in suppressing neuronal activity by increasing the dose of injected bromazepam (Fig. 2*b*). This indicated again a dose-dependent relationship between the effects of GABA and the benzodiazepine derivative.

These electrophysiological findings on vestibular and cerebellar neurones demonstrate for the first time a functional antagonism between benzodiazepine derivatives and the inhibitory neurotransmitter GABA. In both systems, inhibitory GABA influences are suppressed in a dose-dependent manner by these psychotropic drugs, but their effect can be overcome by increasing local GABA concentrations, either through stimulation of the appropriate axons or by microiontophoretic delivery of GABA. By contrast, the effect of the specific GABA antagonist, bicuculline, does not seem to be impaired by benzodiazepine. To ascertain that the benzodiazepine derivative diazepam

Fig. 1 Modification of antidromic (1) potentials recorded from a single neurone in the Deiters nucleus of the cat. *a*, Potential obtained of the antidromic stimulation of the vestibulo-spinal tract with single pulses of 0.5 mA, 0.05 ms duration (five responses superimposed). *b*, Abolition of the antidromic potential by preceding orthodromic stimulation (2) (train of three pulses, 0.8 mA, 0.1 ms, 250 per s) of Purkinje fibres. *c*, Antidromic response obtained 6 min after intravenous injection of diazepam (0.5 mg kg^{-1}) is unchanged. *d*, Suppression of the inhibitory effect of the orthodromic stimulation (compare *b*) after injection of diazepam (5 min).



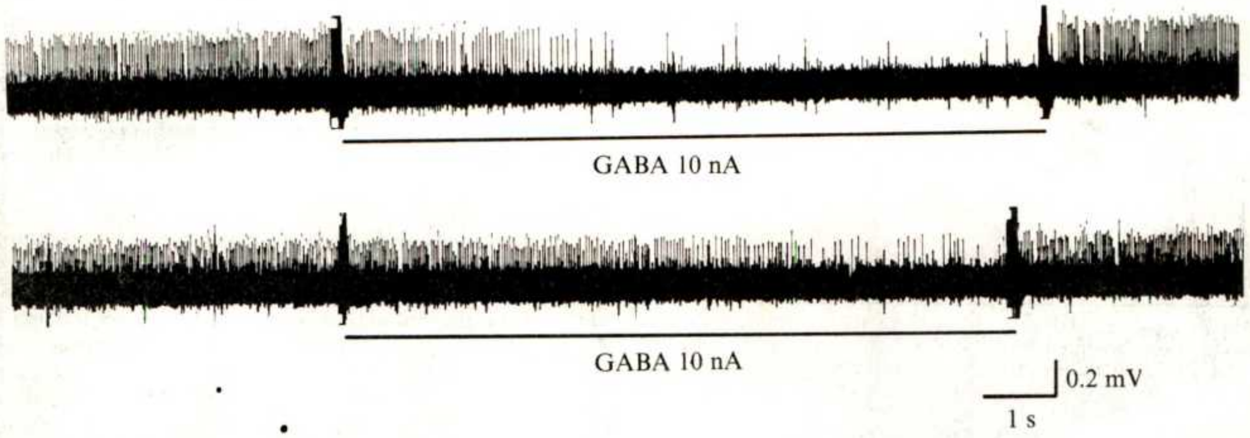


Fig. 2 Single unit activity of a rat Purkinje cell recorded through the central barrel of a five-barrelled micropipette[13]; GABA was delivered from a lateral barrel as a 0.5–1 M solution at *p*H 3.0–3.5. *a*, Delayed inhibitory effect of GABA (10 nA) 47 min after intravenous injection of bromazepam (2 mg kg^{-1}). Action potentials persisting during the second two-thirds of GABA iontophoresis represent the non-suppressible climbing fibre responses[14]. *b*, Further attenuation of the GABA effect (10 nA) 2 min after a second intravenous injection (4 mg kg^{-1}).

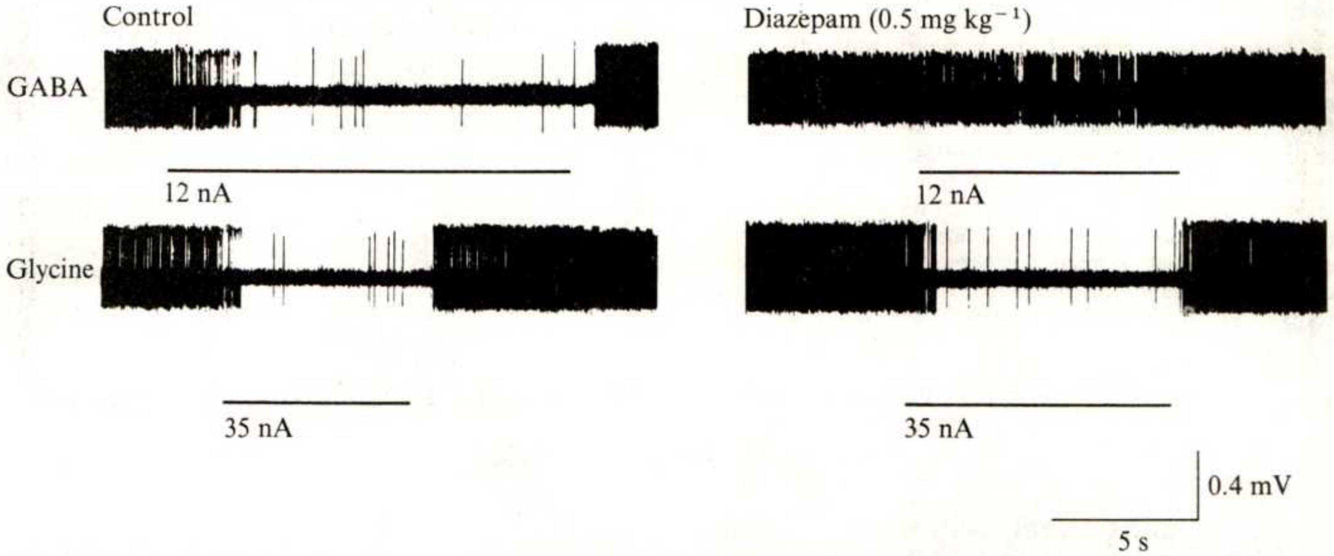


Fig. 3 Single unit activity of a cat Purkinje cell. GABA and glycerine were delivered alternately by iontophoresis (12 nA and 35 nA respectively, indicated by horizontal bars) at *p*H 3.0 from 0.5 M solutions. After intravenous injection of diazepam (0.5 mg kg^{-1}) the GABA depression of cell activity (control) was nearly abolished. In contrast, the glycine-induced depression was not reduced after injection of drug.

specifically counteracts GABA-ergic transmission its effect on glycine-induced inhibition[3] was tested in cat Purkinje cells (Fig. 3). These neurones were less sensitive to glycine and intravenously administered diazepam had no antagonistic influence in the inhibitory effect seen after the iontophoretic delivery of glycine. Thus, this study on Purkinje cells suggests differential effects of benzodiazepines on the two inhibitory amino acids, GABA and glycine. In further experiments, the soluble benzodiazepine derivatives, chlordiazepoxide HCl, was applied iontophoretically to Purkinje cells in rats and cats (unpublished results). Again, the GABA-mediated inhibition of Purkinje cells by basket cells, as well as the direct inhibitory effects of GABA were attenuated or abolished.

In summary, these results fail to support the idea that the action of benzodiazepine could be explained by a facilitation of the GABA-ergic transmission[5,6]. On the contrary, the GABA-mediated inhibition on Deiters neurones and Purkinje cells was clearly antagonised by these drugs.

This work was supported by grants from the Swiss National Foundation for Scientific Research and the Dr Eric Slack-Gyr Foundation, Zürich.

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Received November 24, 1975; accepted January 26, 1976.

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Differentiation of L-aspartate and L-glutamate high-affinity binding sites in a protein fraction isolated from rat cerebral cortex

THE possibility that L-glutamate and L-aspartate are excitatory transmitters in the CNS is supported by neurochemical and neurophysiological evidence[1]. The problems of identifying particular synapses that are excited by one or the other of these dicarboxylic amino acids, and distinguishing

Table 1 Inhibition of the binding of L-^{14}C-glutamate and L-^{14}C-aspartate to the hydrophobic protein from rat cerebral cortex by L-glutamate inhibitors

	Bound L-^{14}C-glutamate (nmol per mg protein)	Bound L-^{14}C-aspartate (nmol per mg protein)
Control	0.56 ± 0.03	1.98 ± 0.28
DL-α-methyl glutamate	0.29 ± 0.04	1.54 ± 0.19
Diethylester of L-glutamate	0.19 ± 0.06	1.46 ± 0.10
L-Nuciferine	0.26 ± 0.09	1.62 ± 0.07

The binding was carried out in aliquots of 100 μg of protein from the first fraction separated by Sephadex LH20 chromatography of the rat cerebral cortex. In the control experiments the binding was carried out with 2×10^{-7} M L-^{14}C-glutamate or 4×10^{-7} M L-^{14}C-aspartate. After incubation for 15 min the samples were rechromatographed on small Sephadex LH20 columns (15 × 0.8 cm) and the bound radioactivity and protein, eluted in chloroform, were measured. For the competition experiments the fractions were preincubated for 30 min with 4×10^{-5} M of the various drugs. The results are the mean of three experiments ± s.d. L-(U)^{14}C-aspartate (231 mCi nmol^{-1}) and L-(U)^{14}C-glutamate (270 mCi mmol^{-1}) were from Amersham.

between the two receptors are, however, more difficult. At the recent International Meeting of Neurochemistry (September 1975) Graham summarised some of the evidence favouring distinct functions for the two amino acids, concentrating on the possible transmitter role of L-aspartate. For example, a higher content of this amino acid is found in the ventral than in the dorsal grey matter of the spinal cord[2], aortic occlusion leads to loss of aspartate content and degeneration of small neurones[3], reduction in aspartate content is seen in the spinal cord after section of dorsal roots[4] and in the olfactory cortex after olfactory bulbectomy[5], which also decreases glutamate. Renshaw cells are slightly more sensitive to the ionotophoretic administration of L-aspartate while the reverse is true of interneurones of the dorsal spinal horn[6]. It is possible that L-glutamate and L-aspartate, being flexible molecules, fold or unfold and could interact with either receptor and McCulloch *et al.*[7] accordingly used kainate, a conformational restricted analogue of glutamic acid, which would be unlikely to fold to occupy the aspartate receptor, and *N*-methyl-D-aspartate, which would be too small to interact with the glutamate receptor (Fig. 1). The difference in sensitivity between the two groups of neurones to these agonists was much greater: the Renshaw cells were much more sensitive to *N*-methyl-D-aspartate and the dorsal interneurones to kainate[7].

The L-glutamate binding proteins of insect[8] and crustacean muscle[9,10] are hydrophobic proteins (that is, proteolipids) extractable with organic solvents. Such proteins did not bind L-aspartate and, in the case of the crustacean muscle, can be separated from another protein binding γ-aminobutyric acid (GABA)[9,11]. Recently we have separated a hydrophobic protein fraction from synaptic membranes of the rat cerebral cortex that binds, with high affinity, L-glutamate and GABA[12]. The same protein fraction also binds L-aspartate and this poses the problem of differentiating between the various binding sites. We shall present here some evidence that this fraction contains a protein binding L-aspartate and which may be its receptor.

Amounts of 200–300 mg of lyophilised cerebral cortex of adult rats were extracted with chloroform–methanol (2 : 1)[13] and the extract, containing 3–4 mg of hydrophobic protein (that is, proteolipid) per g fresh tissue, was chromatographed on a Sephadex LH20 column equilibrated with chloroform. Elution was carried out with chloroform and a series of mixtures of chloroform–methanol of increasing polarity[14]. The first protein fraction, eluted in chloroform in the void volume of the column, is the one that binds L-^{14}C-aspartate and L-^{14}C-glutamate. The binding sites were differentiated by the following experiments. (1) Aliquots of the first protein fraction, containing 100 μg of protein, were preincubated with 4×10^{-5} M of L-glutamate or with 2×10^{-5} M L-aspartate; then they were submitted to binding with 4×10^{-7} M ^{14}C-aspartate or 2×10^{-7} M L-^{14}C-glutamate following an experimental procedure similar to that indicated in Table 1. It was found that L-aspartate did not inhibit the binding of L-^{14}C-glutamate (0.41 nmol per mg protein in the control and 0.43 ± 0.02 in the experimental) and, vice versa, L-glutamate only slightly inhibited the binding of L-^{14}C-aspartate (3.07 nmol per mg protein in the control and 2.62 ± 0.07 in the experimental).

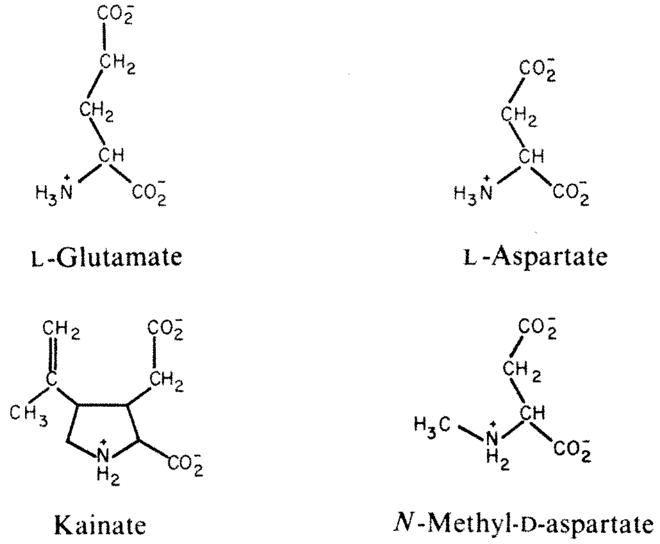

Fig. 1 Structural formulae of L-glutamate and L-aspartate and their respective analogues: kainate and *N*-methyl-D-aspartate.

(2) Experiments were carried out with the physiological competitors of L-glutamate. DL-α-methyl glutamate[15], the diethylester of L-glutamate[15] and L-nuciferine[16] were much more potent in blocking the binding of L-^{14}C-glutamate than in blocking that of L-^{14}C-aspartate (Table 1). The three drugs inhibited, respectively, by 65, 48 and 54% the binding of L-^{14}C-glutamate and only by 26, 22 and 18% that of L-^{14}C-aspartate.

(3) The most striking results were, however, those obtained with the two agonists kainate and *N*-methyl-D-aspartate, which according to McCulloch *et al.*[7], bind preferentially glutamate and aspartate receptors respectively (Fig. 1). Kainate inhibits the binding of L-^{14}C-glutamate by 54% but it does not interfere with the binding L-^{14}C-aspartate (Table 2). On the other hand, *N*-methyl-D-aspartate does not change the binding of L-^{14}C-glutamate but inhibits that of L-aspartate by 45%. These results are in

Table 2 Inhibition of the binding L-^{14}C-glutamate and L-^{14}C-aspartate to the hydrophobic protein from rat cerebral cortex by their analogues kainate and *N*-methyl-D-aspartate

	Control	Kainate (2.5×10^{-5} M)		*N*-Methyl-D-aspartate (4×10^{-5} M)	
Labelled amino acid	(nmol per mg protein)	(nmol per mg protein)	Inhibition %	(nmol per mg protein)	Inhibition %
L-^{14}C-Glutamate (2×10^{-7} M)	0.56 ± 0.03	0.26 ± 0.02	54	0.57 ± 0.11	0
L-^{14}C-Aspartate (4×10^{-7} M)	1.98 ± 0.28	1.99 ± 0.19	0	1.09 ± 0.07	45

The binding was carried out as described in Table 1. The results are the mean of three experiments ± s.d. Kainate and *N*-methyl-D-aspartate were generous gifts from Professor D. R. Curtis.

agreement with the physiological observations of McCulloch *et al.*[7], and support their interpretation[17].

Our findings suggest that the hydrophobic protein fraction isolated from the cerebral cortex, in the void volume of the Sephadex LH20 column, contains different binding sites for L-aspartate and L-glutamate. We have previously shown that it also contains different binding sites for GABA[12]. It remains to be seen whether these binding sites reside in a single or, more probably, in different proteins and whether they represent the true receptors or belong to the active transport system for these amino acids[18,19]. In favour of their possible receptor function is the fact that, in our experiments, the binding is carried out in the absence of Na ions, which are essential for the transport mechanism to operate. Furthermore, several of the drugs used, such as *N*-methyl-D-aspartate, kainate and diethyl ester of L-glutamic acid are not substrates for the transport system[19].

This work was supported by a grant from the US National Institutes of Health.

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Received October 17, 1975; accepted January 22, 1976.

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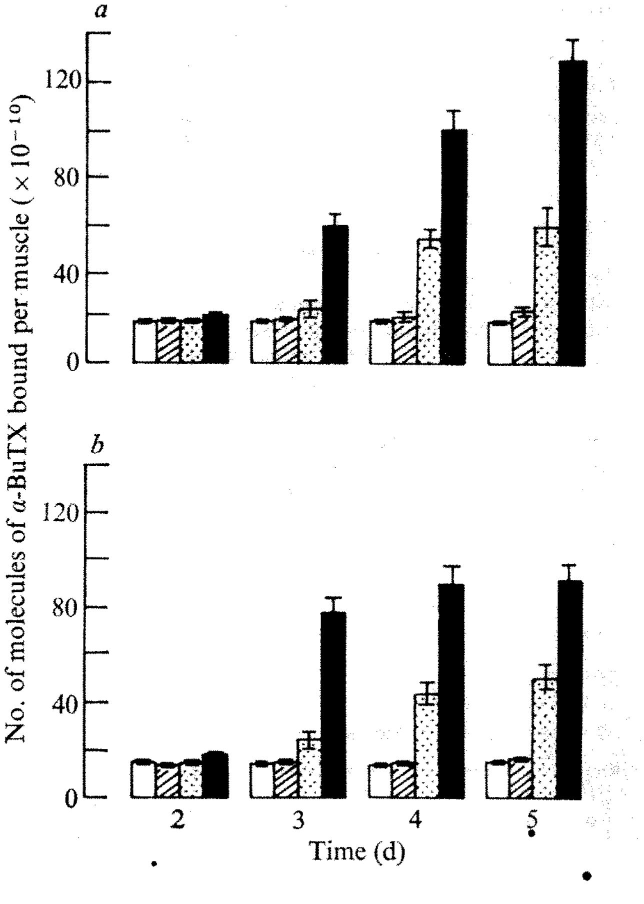
Comparison of α-bungarotoxin binding to skeletal muscles after inactivity or denervation

DENERVATION of skeletal muscles causes a spread of acetylcholine (ACh) sensitivity along muscle fibres, but it is not yet clear whether this results from muscle inactivity or from a loss of trophic influence of the nerve on the muscle[1]. It has been suggested that inactivity is responsible for the phenomenon because the application of a cuff containing a local anaesthetic to a nerve blocked the conduction of the nerve impulses and caused the development of widespread sensitivity to ACh (ref. 2, but see ref. 3). The local anaesthetics used in that study, however, have since been shown to block axoplasmic transport *in vitro*[4,5] and *in vivo*[6], as well as to induce neuronal degeneration[7]. Axoplasmic transport seems to be involved in the trophic influence of the nerve on muscle because blocking this transport induces spreading of ACh sensitivity in muscle fibres[8,9]. We have used tetrodotoxin (TTX), incorporated into silicone cuffs placed around motor nerves, to test whether the resultant inactivity induced a spread of ACh sensitivity in skeletal muscle, as inferred from the binding of α-bungarotoxin (α-BuTX). TTX was chosen because it does not block axoplasmic transport *in vitro*[5].

Female Sprague–Dawley rats (225–250 g) were anaesthetised with ether, the left sciatic nerve was exposed, and a hollow cylindrical cuff made from Silastic 382 (Dow Corning) was placed around the nerve. The control cuffs contained 20% NaCl (w/w) mixed in the Silastic, and the TTX cuffs contained in addition 0.02% TTX (Sigma); each TTX cuff contained approximately 5 μg of TTX. Paralysis was evaluated three times a day by inspecting the toe-spreading reflex when the animals were lifted by the tail[7]; paralysis was present when the animals that received a TTX cuff recovered from anaesthesia and lasted 3–5 d, but the animals that received a control cuff showed no paralysis. The muscles of the contralateral side served as unoperated controls in the animals that received a control cuff, while those contralateral to a TTX cuff were denervated by removing a 5-mm segment of the sciatic nerve in the mid-thigh region.

At various times thereafter, the extensor digitorum longus (EDL) and soleus (SL) muscles from both sides were removed under ether anaesthesia and exposed to ^{125}I-α-BuTX[10]. The muscles were incubated for 1 h (22 °C) in a Krebs solution containing labelled α-BuTX (1 μg ml^{-1}), and then washed for 16–20 h with Krebs solution (4 °C). The total radioactivity retained by the muscles was measured (Nuclear Chicago gamma spectrometer), and the change in α-BuTX binding induced by control cuffs, TTX cuffs and surgical denervation was compared (Fig. 1). The muscle inactivity produced by the TTX cuffs resulted in only a slight increase in α-BuTX binding after 3 d, but α-BuTX binding increased fivefold in the SL and threefold in the EDL muscles denervated for the same time; the difference of α-BuTX binding to the TTX-inactivated

Fig. 1 Effect of TTX cuffs, control cuffs, and surgical denervation on the binding of α-BuTX to the EDL (*a*) or SL (*b*) muscle of the rat. Each column represents the mean ± s.e. of 7–11 muscles. Binding is expressed as molecules of α-BuTX bound, which was calculated from the specific activity of the ^{125}I-α-BuTX used. Columns: open, unoperated; hatched, control cuff; spotted, TTX cuff; black, denervated.



muscles and the denervated muscles was significant ($P<0.001$). But muscles that had been paralysed for 4 and 5 d by TTX cuffs showed a clear increase in α-BuTX-binding ($P<0.001$), although the magnitude of this increase was again less ($P<0.001$) than that produced by denervation. Some of the animals that had been exposed to TXX cuffs for 4 and 5 d showed some recovery of function and it is possible that this short (< 12 h) recovery reversed some of the induced increase of ACh receptors; however, its effect was probably small because α-BuTX binding was still increased (240±30% of control) in the muscles of animals 3-4 d after recovery from 4–5 d of TTX-induced inactivity.

The increased α-BuTX binding observed with the TTX cuffs was not due to nerve degeneration. Rats that had received cuffs 3 d earlier were anaesthetised with pentobarbital (intraperitoneal, 50 mg kg^{-1}), the sciatic nerve was exposed and stimulated electrically (supramaximal voltage, 0.3 ms, 0.4 Hz), and the isometric contractions of the EDL muscle were recorded; the muscle was also stimulated directly. In nine animals in which impulse conduction was completely blocked by a TTX cuff, nerve stimulation distal to the cuff induced a muscle contraction (11.4±0.7 g tension per 100 mg muscle wet weight; mean ±s.e.) similar to that produced by direct muscle stimulation (11.9±0.7 g): thus, TTX, unlike lidocaine[7], does not induce nerve degeneration. In 10 animals that received a control cuff, nerve stimulation proximal or distal to the cuff, and direct muscle stimulation all induced similar muscle contractions (10.8±0.6, 11.6±0.5, 11.5±0.4 g tension, respectively), showing that the cuff itself did not damage the nerve.

Other results demonstrated that the TTX cuffs did not block axoplasmic transport *in vivo*. For these experiments a ligature (Ethicon 4-0 thread) was tied around the sciatic nerve 3–4 mm distal to the cuffs and the accumulation of cholinesterase (ChE) activity at these ligatures after 20 h (ref. 11) was measured. Nerve segments (2 mm) were weighed, homogenised in ice-cold sucrose (0.32 M; 20 mg tissue per ml) and the ChE activity in 50 μl of the homogenate was assayed (22 °C) by the method of Ellman *et al.*[12], using acetylthiocholine (200 μM) as the substrate. There was no significant difference ($P>0.20$) between the accumulation of ChE activity at a ligature distal to a TTX cuff and the accumulation in ligatured control nerves (289±22% of penultimate segment; mean±s.e.), whether the ligature was tied 1 h (306±22%) or 24 h (271±27%) after placing the TTX cuff.

Thus, our results show that local application of TTX to the sciatic nerve of rats will produce muscle inactivity without causing nerve degeneration or blocking axonal transport. This inactivity increases the amount of α-BuTX bound by the SL and EDL muscles, but this change seems to be less than and to have a different time course from that induced by denervation. This difference might reflect a role for factors other than activity in the control of ACh sensitivity in skeletal muscles.

We thank the Medical Research Council of Canada for financial support and Dr W. E. Dennis of Dow Corning Ltd for Silastic 382.

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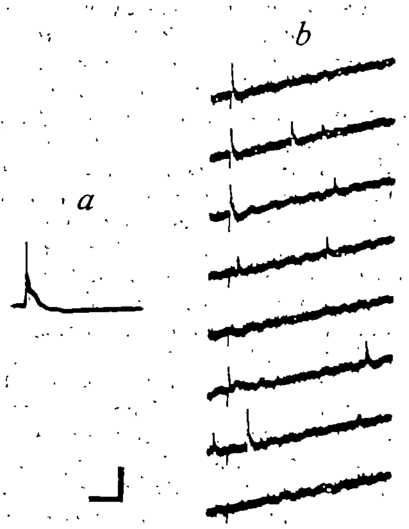
Suppression of transmission at foreign synapses in adult newt muscle involves reduction in quantal content

IN the search for the principles underlying the orderly development of the nervous system, it is interesting to know whether vertebrate neurones or muscle fibres can distinguish synaptic contacts of the axons which normally innervate them from the input of incorrect (or foreign) nerves. This possibility has been considered by asking whether foreign synapses induced on a denervated muscle fibre are displaced or suppressed when the muscle is reinnervated by its normal nerve. Observations of oculomotor reflexes in goldfish after experimental cross innervation of extraocular muscles led to the proposal that correct reinnervation suppressed foreign synapses[1]. Attempts to reproduce these results in the same system[2], however, led to the opposite conclusion—that foreign transmission continued unabated after reinnervation by the correct nerve. A similar persistence of foreign synaptic function was found in reinnervated perch gill muscle[3], frog fast muscle[4], mammalian skeletal muscle[5,6] and sympathetic ganglion cells[7]. Adult salamander muscle is the one other system in which suppression of incorrect synapses has been indicated; by the criterion of muscle fibre contraction monitored during foreign innervation and correct reinnervation, Cass *et al.*[8] concluded that return of the correct nerve caused inactivation of foreign synaptic input. The present investigation examines this phenomenon at the level of transmitter

Fig. 1 Responses from a single anconeus muscle fibre 50 d after correct nerve resection. *a*, Single stimulus to the correct nerve evoked a large endplate potential with action potential superimposed. *b*, Consecutive responses to repeated maximal stimulation of the foreign nerve at a frequency of 0.2 per s. One stimulus pulse occurs near the beginning of each sweep, as indicated by artefact. With some stimuli the foreign nerve failed to release transmitter (traces 4, 5, 7 and 8 from top). Note the spontaneous miniature endplate potentials occurring randomly in (*b*). Calcium concentration normal (1.8 mM) in both. Calibration marks 40 mV and 100 ms in (*a*), 2 mV and 400 ms in (*b*). All five fibres studied in the muscle had been reinnervated by the correct nerve and had low quantal contents for foreign transmission (m = 0.66, 0.94, 1.06, 0.43, 1.47).



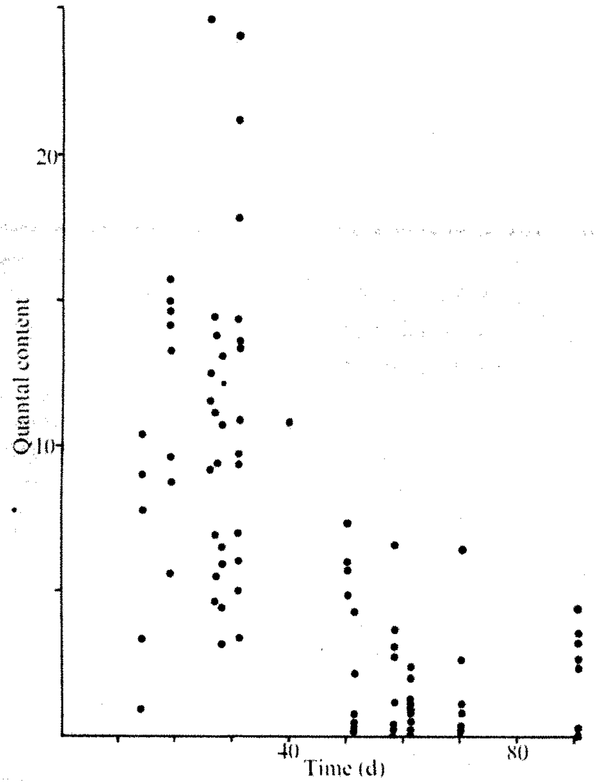


Fig. 2 Scatter diagram of mean quantal content of foreign nerve release plotted as a function of time after the correct nerve was resected. Each point represents the mean content at a single anconeus fibre, measured as indicated in the text. Correct reinnervation was first detected 26 d after resection, and was completed within 50–60 d. In some cases movement artefacts resulting from stimulation of the correct nerve prevented us from determining the absolute amplitude of the correct junctional potential. Because of variability in the rate of correct nerve regrowth, we do not know the time elapsed since correct reinnervation of any given muscle fibre. Not represented in this figure are four muscles, examined at 57, 70, 93, 135 d, in which no foreign nerve responses were found.

release from individual synapses, and provides evidence that transmission by foreign nerve terminals declines in quantal content when adult newt muscle is reinnervated by its correct nerve.

Adult newts, *Notopthalmus viridescens*, were anaesthetised with tricaine (1/1,000 in Ringer solution). The forelimb flexor (humero-antibrachialis) muscle was excised completely and its nerve was displaced laterally to the surface of the antagonistic anconeus muscle. The animals were then held in tanks of water and fed weekly on liver or brine shrimp. Two weeks after the initial operation the correct nerve to the anconeus muscle was crushed, or cut and resected. In some animals the correct nerve was crushed a second time 2 or more weeks after the initial denervation. At various times after the final denervation intracellular recordings were made from single fibres of the anconeus muscle in normal amphibian Ringer, with independent stimulation of the correct and foreign nerves.

The quantal content of the foreign transmission was determined in individual fibres from the coefficient of variation of the evoked responses[9], obtained by stimulating 200 times at a frequency of 0.2 per s. In 25 fibres this value for quantal content was compared with that obtained by the method of failures[9]; there was no significant difference between the values determined by these two means.

Muscles examined within the first week after lesion of the correct nerve showed no functional transmission from either nerve. Between the first and second week transmission from the foreign nerve was detected in a few fibres; in these, stimulation evoked endplate potentials of low amplitude, with frequent failures. The longer the period of correct denervation, the greater was the extent and efficacy of foreign innervation. Thus, when the correct nerve was cut rather than crushed initially, or when it was crushed a second time 10–14 d after the first denervation, a greater proportion of the fibres developed functional foreign transmission and the average amplitude of the responses evoked by foreign nerve stimulation was larger. In some fibres the amplitude of foreign response became large enough to cause an action potential and twitch, like that seen in normally innervated adult fibres[10,11].

Approximately 2 weeks after the correct nerve was crushed, or 3–4 weeks after it was resected, its axons began to reinnervate the muscle. At this stage individual fibres were found which received functional contact from both correct and foreign axons. The correct synapses rapidly developed to their normal state of transmission since correct input, when detected, was almost always sufficiently strong to cause normal action potentials (Fig. 1*a*). Within 3 months after the final denervation all fibres had received correct reinnervation.

The synaptic potentials resulting from foreign transmission were usually subthreshold in those fibres which had received correct reinnervation (Fig 1*b*). The mean quantal release from the foreign nerve was measured in individual fibres at various times after correct nerve resection. The pooled results from 84 fibres of 13 muscles in which the correct nerve had been resected or crushed twice are presented in Fig. 2. These data reveal that foreign transmission declines in quantal content with time after the correct nerve returns. A similar series of experiments in which the correct nerve was crushed only once gave the same general result, although the maximum values of foreign quantal content were lower and the decline occurred more rapidly.

Although correct reinnervation resulted in a decline in the amount of transmitter released per impulse from foreign terminals, the average size of the quantal events remained unchanged; release failure was frequent yet the mean amplitude of foreign evoked quanta was normal (0.5–2 mV) and equal to that of spontaneous potentials in the same fibre, as illustrated in Fig. 3 (presumably the spontaneous events result from release by correct and foreign terminals). This indicates that the suppression of foreign inputs involves a decline only in the number of packets of transmitter released, and not in the amount of transmitter per packet nor in the sensitivity of muscle membrane to transmitter at the site of foreign nerve contact.

One potential difficulty with such physiological analysis arises from the fact that not all the fibres in the anconeus muscles can be assumed to receive innervation from foreign axons. Thus, after correct reinnervation a complete lack of response to foreign nerve might result either because a fibre had never received foreign innervation or because such innervation had been suppressed. But this does not seem to present a real problem in our experiments: in the set of animals with correct nerve resection, all muscles ($n=10$) examined in the first 2 months had some fibres with foreign transmission whereas in only half (4/8) of those studied 2–4 months after denervation could fibres with foreign transmission be found.

In six animals the correct nerve was recrushed 6 months after the first lesion; foreign transmission reappeared within as little as 2 days, and developed sufficient strength to elicit action potentials within 1 week. This rapid return of foreign transmission, as compared with the 10–14 d delay preceding the initial establishment of foreign innervation, suggests that foreign synapses, suppressed on correct reinnervation, may remain in close proximity with the muscle fibres.

Neither the morphology nor the relative position of correct and foreign synapses along individual muscle fibres is known. In normal muscles single fibres are innervated

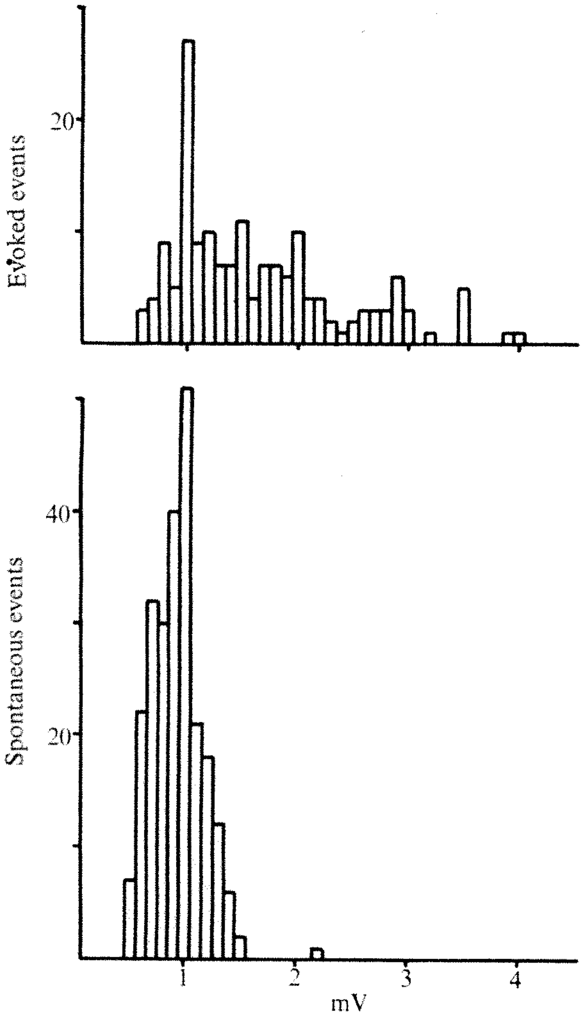


Fig. 3 Histograms comparing the amplitudes of spontaneous miniature endplate potentials (top) and of responses evoked by foreign nerve stimulation (bottom) in the muscle fibre from which examples in Fig. 1 are taken. The foreign nerve was stimulated 251 times, with 87 failures: $m = 1.1$. The calcium concentration was normal (1.8 mM). Noise level, 100 μV.

at several sites along their length, as revealed by electrophysiology[10,11] and by cholinesterase staining (unpublished). This suggests that here correct and foreign synapses occur in close proximity to one another.

Our results indicate that muscles of the adult newt differ from those of higher vertebrates in that they can discriminate between correct and incorrect innervation, and suppress the incorrect synaptic input. Urodele amphibia, with the ability to regenerate limbs, may retain in adulthood a capacity usually confined to embryos which permits them to correctly reconnect their peripheral innervation.

This work was supported in part by a training grant from the Sloan Foundation and a grant from the National Institutes of Health.

Note added in proof: The proportion of anconeus fibres with functional foreign synapses has been determined at short and long periods after correct nerve resection. In eight muscles examined 19–47 d after resection all of 176 fibres received foreign input, whereas in nine muscles 57–135 d after resection only 37 of 151 fibres (25%) had functional foreign input.

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Received December 8, 1975; accepted January 2, 1976.

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Effect of muscle disuse on acetylcholine receptors

MOTOR nerves have an important rôle in the trophic regulation of many properties of skeletal muscle, including acetylcholine receptor (AChR) distribution[1]. In innervated muscle, the density of AChR at neuromuscular junctions is at least 2,500 times greater than at extrajunctional regions[2]. When the motor nerves are sectioned, extrajunctional AChR density increases markedly[2–5]. The trophic mechanisms by which the nerves normally prevent this denervation change are not yet well understood. It has been suggested that muscle use contributes to the control of AChR. This hypothesis has been tested in two ways. First, electrical stimulation was applied directly to denervated muscle, and the extrajunctional ACh sensitivity was determined. The results indicated that stimulation-induced use largely prevented the denervation effect[6,7]. Second, attempts have been made to assess the effect of disuse of muscle on extrajunctional ACh sensitivity. Various methods have been used to eliminate muscle contraction but all have had shortcomings. In some cases, the 'disuse' has been incomplete[8–10]; in others, the methods used to block nerve conduction[7,11] have produced blockade of axonal transport[12,13], which may itself result in denervation effects[14,15]. Using a new model of disuse free of these problems, we have now compared the effects of disuse and denervation with respect to extrajunctional AChR density. We report here evidence that disuse produces a significant increase in extrajunctional AChR, but does not completely reproduce the effect of surgical denervation.

In our model, complete blockade of nerve conduction, without alteration of other nerve functions, has been produced by repeated local application of tetrodotoxin (TTX) to the sciatic nerves of rats. TTX interferes with conduction of electrical impulses in excitable membranes by blocking sodium conductance channels[16]. Quantitative determinations of extrajunctional AChR density in disused and denervated muscles have been made by means of a technique using ^{125}I-α-bungarotoxin binding[17].

Experimental procedures were carried out on Sprague–Dawley female rats (190–215 g) under chloral hydrate anaesthesia (400 mg kg^{-1}). The sciatic nerve was exposed by an incision in the thigh, and a subperineurial injection of TTX or Ringer solution was made by means of a glass micropipette coupled to a micrometre-driven syringe, mounted on a micromanipulator. In initial trials, we found that intraneural injection of 2 μg of TTX in 2 μl of Ringer solution produced complete paralysis beginning within 10 min, and lasting usually for more than 48 h. More detailed studies of the physiological effects of the TTX treatment were made in six clinically paralysed animals 48 h after intraneural injection. To determine whether the nerve block was complete, the sciatic nerve was stimulated *in vivo* proximal to the injection site, while electrical recordings were made at high gain from the surface of the soleus and extensor digitorum longus (EDL) muscles, and the exposed muscles were observed under the stereomicroscope. The nerve and muscle were then removed in continuity, and intracellular recordings were made *in vitro* (during nerve stimulation) with conventional 3 M KCl-filled microelectrodes. By these criteria, nerve conduction was totally blocked at the level of the TTX injections, although the distal portion of the nerve and the muscle functioned normally (see below).

In the experiments reported here we therefore adopted a uniform protocol in which the injections were repeated at 48-h intervals. Just before each repeat injection of TTX and at the termination of each experiment, blockade was tested by nerve stimulation. When blockade was incomplete the animals were discarded.

At 0, 4, and 7 d, the rats were anaesthetised and the EDL and soleus muscles from each were dissected out and prepared for measurement of extrajunctional AChR density. This measurement depends on the specific, quantitative and irreversible binding of ^{125}I-α-bungarotoxin with AChR. The number of bungarotoxin-binding sites is proportional (perhaps equal) to the number of AChR sites[18].

The results (Table 1) show that disuse produced a significant increase in extrajunctional AChR in all groups. Denervation always produced a significantly greater increase in AChR than did disuse. The disused soleus showed significantly greater increase in extrajunctional AChR than did the comparable EDL at both 4 d ($P < 0.01$) and 7 d ($P < 0.05$).

The injection procedure itself could not account for these effects since subperineurial injections of Ringer solution did not produce an increase in AChR density above control levels. TTX did not exert an inhibitory influence on extrajunctional receptors, since treatment with denervation plus TTX resulted in levels of AChR similar to those of denervation alone.

The effects of the experimental treatments on the morphology and function of the sciatic nerve were evaluated. Nerves of all the 7-d TTX and Ringer-injected animals were embedded in Epon, and 1-μm thick sections, stained with toluidine blue, were coded and examined microscopically. No morphological differences between TTX and Ringer-injected nerves were found; in both only occasional degenerated axons were identified. The distal segment of the blocked nerves and the muscle fibres could function normally, since stimulation distal to the TTX block resulted in brisk muscle contraction. Intracellular recordings of minature endplate potentials showed normal frequency (soleus=2.0±0.18 s^{-1}; EDL=2.4±0.27 mV) and amplitude (soleus=0.37±0.03 s^{-1}; EDL=0.46±0.05 mV, corrected[19] to 75 mV RMP), indicating that the motor nerve terminals remained intact. Fast axonal transport of protein and glycoprotein was measured after injection of ^{3}H-leucine or ^{3}H-fucose into the lumbar ventral horns. In nerves treated with TTX for 7 d the rate through the level of injection was normal (385±34 mm d^{-1})[20] and equal to that of the control side. Delivery of ^{3}H-fucose-labelled glycoproteins to the motor nerve endings was also similar in control and TTX-treated nerves, as shown by autoradiography of hindfoot lumbrical muscles 24 h after labelling of the ventral horns[21].

Table 1 Effects of treatment on extrajunctional AChR density

Treatment		Time	
		4 d	7 d
Soleus muscle			
Untreated	22±2 (30)†		
Denervation		195±16 (11)*	381±39 (14)*
TTX		96±23 (5)*†	243±46 (7)*§
Ringer		22±1 (3)†	12±3 (6)†
TTX+denervation		200±23 (5)*	
EDL muscle			
Untreated	10±1 (36)†		
Denervation		168±26 (11)*	356±35 (13)*
TTX		15±4 (5)†‡	130±33 (7)*†
Ringer		6±1 (3)†	7±2 (6)†
TTX+denervation		141±29 (5)*	

*Greater than normal $P < 0.001$.

†Less than denervation $P < 0.005$.

‡Greater than normal $P < 0.05$.

§Less than denervation $P < 0.02$.

Muscles were teased longitudinally into three or four pieces, pinned at resting length and incubated for 3–4 h in modified Ham's F-12 culture medium containing 0.2–0.3 μg ^{125}I-α-bungarotoxin per ml (specific avtivity 2–5×10^4 (Ci mol^{-1}). The muscles were then washed thoroughly, fixed in 4% glutaraldehyde, and neuromuscular junctions were stained for acetylcholinesterase[25]. Junction-free muscle strips were further dissected under the stereomicroscope into small bundles of 10–100 fibres. The number and length of fibres in each bundle were determined and the radioactivity was measured in a gamma counter. Frozen muscle sections stained with haematoxylin and eosin were used to compute average fibre diameters. Results are expressed as AChR per $μm^2$ (mean±s.e.m.). Numbers in parentheses indicate number of muscles.

Based on these observations, the TTX-blocked preparation seems to fulfil the requirements of a pure disuse model. Nerve impulses were effectively blocked throughout the experimental period, thereby eliminating all nerve-mediated muscle usage. The experimental procedure did not damage the nerves structurally, interfere with spontaneous transmitter release, or block axonal transport and delivery.

The observation of a denervation-like increase of extrajunctional AChR in disused muscles is generally consistent with previous reports[8–10]. Our quantitative studies demonstrate that the effect of disuse is large, but is not as great as that of denervation. This implies that some additional neurotrophic influence also contributes to the regulation of AChR. Many possible factors have been postulated, including substances carried by axonal transport[14,15], spontaneously released ACh[22], and nerve–muscle membrane interaction[23,24] but further studies are needed to clarify the role of each. We conclude that use has a prominent but not exclusive role in the neurotrophic regulation of extrajunctional AChR.

This work was supported by grants from the National Institutes of Health, the Dysautonomia Foundation and the Muscular Dystrophy Association of America.

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Received December 1, 1975; accepted January 16, 1976.

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Phospholipid exchange proteins in synaptosome and myelin fraction from rat brain

ACETYLCHOLINE stimulates the turnover of phosphatidylinositol (PI) and phosphatidic acid (PA) in brain *in vitro* and *in vivo*[2,3]. It has been suggested that this effect on phospholipid metabolism is related to synaptic nerve pulse conductance[4,5], and that phospholipid exchange proteins transfer PI from the site of synthesis—the endoplasmic reticulum—to the synaptosomal plasma membrane[5]. Exchange proteins have been isolated from bovine brain that transfer PI to membranes deficient in this phospholipid *in vitro*[6-8]. These proteins also catalyse the transfer of phosphatidylcholine (PC) and PA, although to a lesser extent[7]. We report here the presence of phospholipid exchange proteins in the soluble protein fractions derived from a synaptosomal membrane and myelin fraction.

Myelin and synaptosomes were prepared from rat brain cerebral hemispheres according to a slightly modified centrifugation scheme used by Morgan *et al.*[9] (legend to Fig. 1). The myelin fraction consisted mainly of irregularly formed multilamellar structures of vesicular appearance, often enclosing remnants of axonal contents (Fig. 1*a*). Electron micrographs of the synaptosomal fraction showed a rather homogeneous preparation of nerve-ending particles containing the characteristic synaptic vesicles and mitochondria; myelin fragments were absent (Fig. 1*b*).

The identity of the membrane fractions was further established by analysis of free cholesterol and total lipid phosphorus in the lipid extracts (Table 1). The values expressed in mg per mg protein compare favourably with those reported by Cuzner *et al.*[15] for myelin (0.460 mg of cholesterol and 0.868 mg of phospholipid) and Lapetina *et al.*[16] for synaptosomes (0.172 mg of cholesterol and 0.450 mg of phospholipid). The phospholipid–cholesterol molar ratio of 1.13 for myelin and 0.55 for synaptosomes agree with reported values[17]. Lipids from myelin and synaptosomes were further identified by two-dimensional, thin-layer chromatography[18]. Only the lipid extract from myelin contained cerebrosides and sulphatides. This confirms that the synaptosome fraction is devoid of myelin, as the electron micrographs indicated.

Soluble proteins were released from the synaptosome and myelin fractions by resuspending the fractions in 1 mM phosphate buffer, *p*H 7.1. After centrifugation at 105,000*g* for 2 h, approximately 15% of synaptosomal protein and 6% of myelin protein were recovered from the membrane-free supernatants. These soluble protein fractions were assayed for phospholipid transfer activity (Table 2). Compared with the crude 105,000*g* supernatant from total brain, the PI and PC transfer activity per mg of soluble protein was enriched eight- and fourfold, respectively, in the synaptosomes, and seven- and twofold in the myelin.

Because the transfer activities in the synaptosomal supernatant may not be representative of most activities found in the 105,000*g* supernatant, activities were compared by isoelectric focusing. The synaptosomal soluble protein fraction contains two major activity peaks at *p*H 5.02 and 5.34 (Fig. 2). Two major activity peaks were also observed with the 105,000*g* supernatant at *p*H 5.05 and 5.30. Each of the four peaks contained PI and PC transfer activity in a PI/PC activity ratio of approximately 2. Since the relative proportion which gave the two activity peaks was similar in both cases, the major phospholipid exchange proteins in the synaptosomes seem to be identical to those in the total brain cytosol. The relatively high transfer activities in the

Table 1 Phospholipid and cholesterol content of membrane fractions

Lipid class	Myelin	Synaptosome
Phospholipid	0.835 ± 0.048 (4)	0.495 ± 0.026 (3)
Cholesterol	0.427 ± 0.069 (4)	0.136 ± 0.008 (3)
Cholesterol* Phospholipid	1.13 ± 0.12	0.55 ± 0.01

Lipid extracts were prepared according to a modified Folch procedure[12]. Lipid phosphorus and membrane protein concentration were measured as before[13]. Cholesterol was determined by the method of Zak *et al.*[14]. Values are expressed in mg per mg protein. Quantities of phospholipid were obtained by multiplying the value of lipid phosphorus by 25. Numbers are given ± s.d. Numbers of experiments are given in parentheses.

*Molar ratio.

Fig. 1 Electron micrographs of the myelin (*a*) and synaptosome (*b*) fraction. Myelin sedimented at 1,000*g* for 15 min was resuspended in sucrose–EDTA–phosphate buffer; the suspension was centrifuged at 1,000*g* for 5 min followed by centrifugation at 1,000*g* for 15 min. The latter pellet was resuspended, layered on 8.5% Ficoll–0.32 M sucrose, and centrifuged at 95,000*g* for 60 min in the Beckmann SW-27 rotor. The myelin fraction was collected at the 8.5% Ficoll interphase. The crude mitochondrial fraction containing the synaptosomes was sedimented and washed three times at 7,500*g* for 25 min (ref. 10). The synaptosomes that collected at the 9–12% and 12–16% interphases of the Ficoll gradient, were pooled. The membrane fractions were prepared for electron microscopy as described before[11].

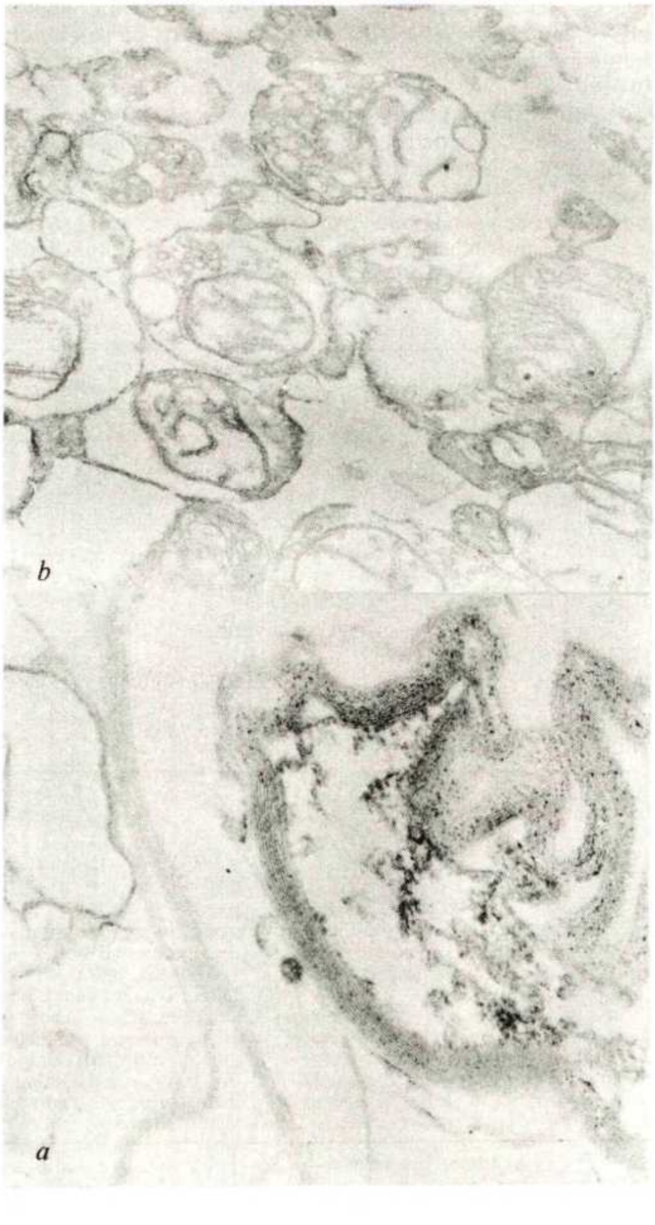


Table 2 Phosphatidylinositol and phosphatidylcholine transfer activities in membrane fractions and cytosol from rat brain

Protein fraction	PI transfer (%)*	PC transfer (%)*
Synaptosomal	28.2±4.2	7.6±1.5
Myelin	25.5±6.5	4.4±0.1
105,000*g* supernatant	3.7±0.4	2.0±0.1

PI and PC transfer activities were determined as before[6,13]. The assay systems consisted of rat liver microsomal donor membranes (1.25 mg of protein) and liposomal acceptor membranes (1 μmol of egg PC, 0.02 μmol of egg PA, 0.01% (by weight) of ^{3}H- or ^{14}C-cholesteryl oleate). The microsomal membranes were labelled specifically with methyl-^{14}C-PC or inositol-^{3}H-PI. Activity is expressed as percentage of the microsomal radiolabelled phospholipid pool transferred to the liposomes, per 30 min per mg of protein at 37 °C.
*Numbers ± s.d. are based on three experiments.

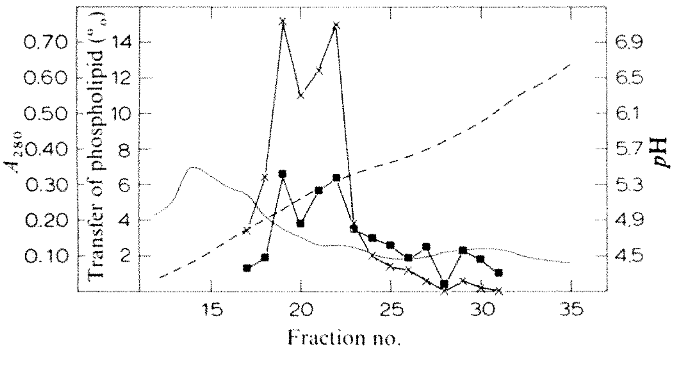
synaptosomes indicate that phospholipid exchange proteins are probably intrinsic to these membrane structures.

Some nerve impulses are propagated through the synaptosomal plasma membranes by triggering the release of acetylcholine at the presynaptic site[20]. *In vitro*, synaptosomes were found to respond to the addition of acetylcholine by an increased synthesis of PA and PI[21,22]. *In situ*, however, the effect on PI was observed throughout the neuronal cell[23,24]. This may be due to phospholipid transfer activities, present in the neurone, which would mask any localised effect by continuously distributing PI for example between the subcellular membrane structures (see also ref. 5).

Synaptosomal plasma membranes cannot synthesise their constituent phospholipids[25]. It is not known to what extent the turnover of PI in this membrane is associated with the transport of this phospholipid from its site of synthesis—the intraterminal smooth endoplasmic reticulum and/or the endoplasmic reticulum in the nerve cell body down the axon. It is assumed that phospholipid exchange proteins are involved and have a role in maintaining the phospholipid composition of a membrane[8].

The transfer activity in the soluble fraction from myelin suggests that exchange proteins are present in the axoplasm, part of which is preserved during the isolation of the myelin fraction. These proteins could be involved in the transport of phospholipids from the neuronal cell body to the nerve endings. Axonal transport of phospholipids has been demonstrated in rabbit nerves[26]. Studies with the dorsal root ganglion of the frog have provided evidence for simultaneous axonal movement of PC and protein[27]. On the other hand, part of the transfer activity may have originated from glial cytoplasm occluded in the myelin fraction.

Fig. 2 Separation of the phospholipid transfer activities present in synaptosomes, by isoelectric focusing. An LKB 8101 electrofocusing column and LKB ampholine solutions (LKB Productor AB, Stockholm) were used according to Vesterberg and Svensson[19]. Electrofocusing of soluble synaptosomal protein (3.7 mg) was carried out at 5 °C with a *p*H gradient from 4 to 8 during 5 h at 200 V followed by 64 h at 500 V; 2-ml fractions were collected. PI (×) and PC (■) transfer activity are expressed as the percentage of the microsomal radiolabelled phospholipid pool transferred per 60 min per 0.2 ml at 37 °C.



The occurrence of identical PI transfer proteins in bovine brain, heart and liver suggests that their action is not uniquely tied to processes occurring in the neuronal cell[7].

We thank Dr A. Verkley for help with the electron microscopy and Dr G. M. Helmkamp for advice.

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Independence of Moloney virus-induced cell-surface antigen and membrane-associated virion antigens in immunoselected lymphoma sublines

THE nature of virus-determined cell-surface antigens (CSAs), recognised by the syngeneic host on neoplastic cells induced by RNA viruses has not been properly defined in spite of much recent speculation about the possible role of cell membrane associated virion proteins[1]. Lymphoma cells induced by the Moloney leukaemia virus (MLV) are said to carry MCSAs. It is a matter of some debate whether MCSA is identical with the tumour-associated transplantation antigen (TATA) detected *in vivo* by the rejection of small tumour grafts in syngeneic preimmunised animals. Views are divided on the origin and control of CSA and/or TATA: some authors regard them as virally induced, but distinct from virion antigens (see for example refs 2–5) whereas others believe that they are virion components, inserted into the cell membrane[6,7]. Antisera against purified virion components (designated according to the recently adopted nomenclature[8]) have been used to demonstrate that p30 and gp69/71 are present on the surface of mammalian type C RNA virus-infected cells[9–11]. We have confirmed this in the present paper using an MLV-

Table 1 Selection history of the YAC462-IR line

Passage no.	Selection: *In vitro**	Selection: *In vivo*	Inoculum: no. of cells	Cytotoxic sensitivity
1–3	Exposure to mouse	Inoculation to	10^5	0.74–0.83‡
4–6	syngeneic/anti-	preimmunised	10^5	0.37–0.64
7–8	MCSA serum and	mice†	10^4	0.24–0.34
9–11	rabbit complement		10^3	0.13–0.27
12–13	before each passage		10^3	0–0.10

The starting material was the YAC ascites line, 462nd serial passage.

*Mouse anti-MCSA serum was produced by the inoculation of 5×10^6 YAC cells irradiated with 6,000 r. to (A × C57 leaden) F_1 mice; 4–6 inoculations were given and serum was collected 1 week after the last inoculation. 5×10^6 cells were incubated with 0.1 ml of a 1:5 dilution of antiserum for 30 min at 37 °C. Antiserum was removed by centrifugation and 0.1 ml of a 1:5 dilution of rabbit complement added. Following further incubation for 45 min at 37 °C cells were counted with Trypan blue staining and inoculated to syngeneic A or (A × X) F_1 hybrids.

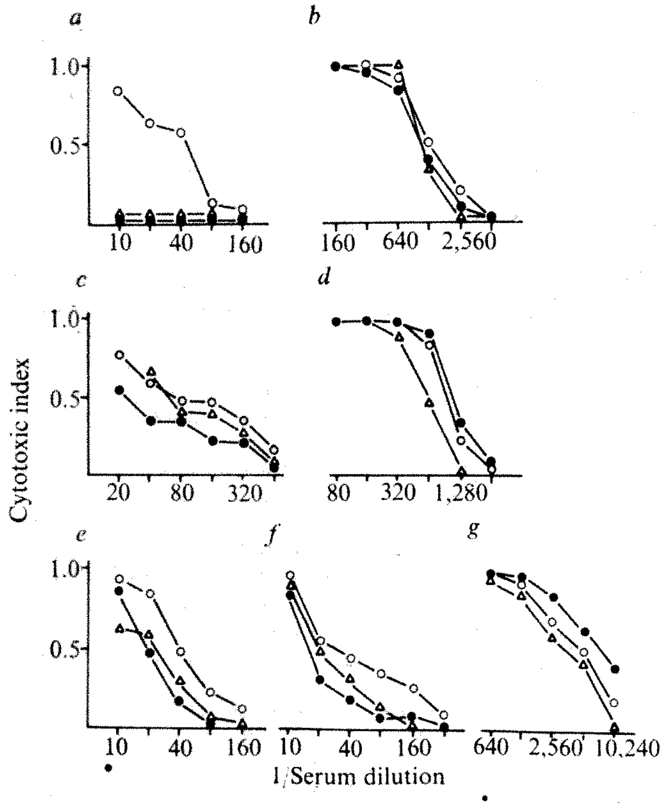
†The immunisation schedule was the same as for antiserum production.

‡A cytotoxic index was calculated by subtraction of the percentage of negative cells in the serum-treated sample from the percentage of negative cells in the control samples divided by the latter figure. The percentage of negatives in control serum ranged from 80 to 100%.

induced mouse lymphoma (YAC) and shown, in addition, that antigen(s) identical with or related to p15 and p12 are also expressed on the outer cell surface. Since these specificities were all detected with antisera produced in foreign species, the question as to which, if any of them, is responsible for MCSA, the antigen recognised by the syngeneic mouse—as mentioned above—remains open. We have approached this question by exposing the YAC lymphoma repeatedly to the immunoselective pressure of anti-MCSA sera and passage through preimmunised syngeneic hosts, until all detectable MCSA has disappeared. The immunoresistant sublines were compared with the original YAC line with regard to the expression of the various virion antigens. The results support that MCSA differs from the known virion antigens, partly or completely.

The combined *in vitro* and *in vivo* selection of the first immunoresistant subline (YACIR) was reported earlier[17]. Selection involved repeated exposure to mouse anti-MCSA sera in the presence of complement, before each passage *in vivo*. The sera were produced in A × C57BL or A × C57L F_1 mice, 3–6 immunisations with 6,000 r. irradiated YAC cells. After each cytotoxic exposure, surviving cells were passaged in syngeneic or semisyngeneic (A F_1) hosts, preimmunised with one inoculation of 6,000 r. irradiated YAC cells 2–2 weeks earlier. After ten such passages, the YACIR subline was completely resistant to the cytotoxic effect of the MCSA antiserum. Quantitative absorption experiments showed that MCSA was still present, but its concentration had decreased tenfold.

Fig. 1 Cytotoxic sensitivity of YAC (○), YACIR (●) and YAC 462-IR cells (△) to the antisera as indicated. *a*, Mouse anti-MCSA; *b*, rabbit anti-mouse; *c*, rabbit anti-FVp15; *d*, rabbit anti-FVgp 71; *e*, goat anti-RLVp30; *f*, goat-RLVp12; *g*, goat anti-RLVgp69/71.



For the present study, we have repeated the immunoselection experiment and obtained a second, independent immunoresistant subline (Table 1). Designated YAC 462–IR, it was completely resistant against the cytotoxic effect of the standard anti-MCSA serum.

For the cytotoxic tests, we used a microassay in which the serial serum dilutions in 0.002-ml volumes were injected under paraffin oil into rings of microtrays (Møller-Coats A/S, Norway) with an automatic Hamilton syringe. Cells (10^3 in 0.001 ml) were injected into the antiserum drop and the plates incubated for 20 min at 37 °C. Subsequently, 0.001 ml rabbit complement was added. The final dilution of complement was 1: 40 for YAC, RBL-5 and FLC, 1: 20 for YACIR, 3T3 and JLS-V9 cells, 1: 8 for YAC462-IR and 1 : 80 for A lymph node cells. Plates were incubated further for 45 min. Thereafter 0.0005 ml Trypan blue was injected into the same drop and the results read under an inverted microscope.

The goat anti-RLV (Rauscher leukaemia virus) sera were kindly provided by: Dr D. C. Fish, (anti-p30); Dr J. R. Stephenson (anti-p12) and Dr M. Strand (anti-gp69/71). The rabbit antisera against Friend virus proteins were kindly provided by Dr D. P. Bolognesi, (anti-p15) and Dr W. Schäfer (anti-gp71).

Figure 1*a* shows the high sensitivity of the original YAC line to anti-MCSA serum in sharp contrast against the complete resistance of the YACIR and YAC462-IR sublines. All three lines were equally sensitive to a rabbit anti-mouse serum (Fig. 1*b*) (raised by hyperimmunising a rabbit with mouse tumour cells induced by methylcholanthrene). Moreover, all lines were about equally sensitive to all tested reagents against the following purified virion components: p30, p12 and gp69/71 prepared from RLV (Fig. 1*e*–*g*); p15 and gp71 prepared from FV (Fig. 1*c* and *d*).

None of the antisera reacted with a series of control mouse cells, including normal A lymphocytes and the cell lines, NIH Swiss/3T3, BALB/3T3 and JLS-V9 (Fig. 2). The specificity of the anti-p30, anti-p15, anti-p12 and anti-gp71 sera was further confirmed by inhibition experiments with the corresponding purified protein (Table 2). These results suggest that MCSA is not identical with the p30, p15, p12 and gp71 specificities, expressed equally on the surface of YAC cells and the two IR-sublines.

It has been suggested that p30 is the principal cell surface antigen recognised by the rat[13–15]. Geering *et al.*[16] have shown that the rat can recognise "group specific" antigens

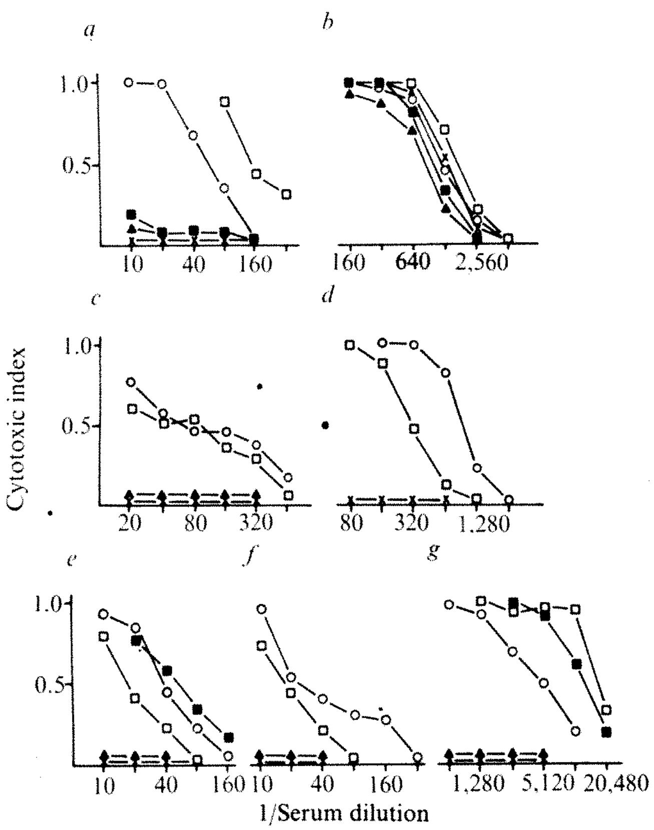


Fig. 2 Cytotoxic sensitivity of lymph node cells (×) of normal A mice, JLS-V9 cells (▲) and 3T3 cells (▲) of NIH Swiss and BALB/c origin, against YAC (○), RBL-5 (□) and F-765 (■). RBL-5 is an RLV-induced leukaemia cell line of C57BL origin and is grown *in vitro* as a suspension culture. F-745 was established *in vitro* as a suspension culture from the spleen of a DBA/2 mouse infected with FV. *a–g*, As for fig. 1.

(p30 in modern terminology) on the surface of leukaemia cells induced by mouse leukaemia virus, whereas the same antigen(s) remain unrecognised in the natural murine host[17].

It was shown recently that p15 is present on the virion envelope[18] and behaves mainly as an interspecies specific antigen. This antigen is recognised by the mouse and may even induce naturally occurring antibodies[17,19]. Both MCSA and TATA are leukaemia virus strain specific, however, in contrast to what has been claimed for p15 and in line with our findings that dissociate p15 clearly from MCSA.

In conclusion, the present work shows that repeated immunoselection, alternating between *in vitro* cytotoxic treatment with anti-MCSA sera, in the presence of complement, and passage through preimmunised mice, leads to immunoresistant sublines. Such lines are resistant to anti-MCSA sera, but their sensitivity to the cytotoxic effect of antisera directed against p12, p15, p30 and gp69/71 virion antigens remained unchanged. This suggests that the syngeneic murine host recognises a virally determined surface antigen, MCSA, that differs from known virion antigens partly or completely.

Table 2 Inhibition of cytotoxicity of antisera with the corresponding virion protein

Serum	Protein	Amount needed for 100% inhibition (ng)	
		RBL-5	YAC
Goat anti-RLVp30	RLVp30	72	36
Goat anti-RLVp12	RLVp12	100	100
Rabbit anti-FVp15	FVp15	125	62.5
Rabbit anti-FVgp71	FVgp71	125	125

The inhibition assay was carried out in the microcytotoxicity assay. The proteins were diluted in antiserum, at dilutions giving 70–90% killing. Ten twofold dilution steps of the proteins in 2 or 3 dilutions of antiserum were included in each test. The cells and complement were added to the drops containing the antiserum–protein mixtures. None of the proteins (500 ng) inhibited the cytotoxic activity of a rabbit anti-mouse serum.

We thank Dr R. Herberman for the RBL-5 cells and Dr C. Friend for the F-745 cells, and Mrs Margareta Hagelin, Miss Irma Jansson and Mrs Maj-Lis Solberg for technical assistance. This work was supported by the Virus Cancer Program of the US National Cancer Institute, the Swedish Cancer Society and King Gustav V's Jubilee Fund.

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Association of Epstein–Barr viral genomes with American Burkitt lymphoma

THE African form of Burkitt lymphoma (BL) is consistently associated with Epstein–Barr virus (EBV), as judged from the presence of elevated serum titres against viral antigens, and the occurrence of EBV DNA and EBV-associated nuclear antigen (EBNA) in the tumour cells[1,2]. It has been postulated that EBV has a role in the causation of BL in Africa. It has also been suggested that aetiological cofactors contribute to the high tumour incidence in the endemic areas. In particular malaria has been proposed by Burkitt[3] because of the geographical coincidence of BL and holo- or hyperendemic malaria. Rare cases of BL occur outside Africa but have not been shown to be associated with EBV[4]. We now present evidence for such an association.

We have studied 12 American patients with the diagnosis BL at the National Cancer Institute, for whom both sera and tumour tissue were available. Sera were assayed for antibody titres to several EBV-associated antigens. Tumour samples were transported frozen to Stockholm for assays of EBV DNA by nucleic acid hybridisation, using ^{32}P-labelled complementary RNA (cRNA) according to Lindahl *et al.*[2].

Among these 12 sera, two (R.A. and R.B.) had an antiviral capsid antibody (VCA) titre of ≥ 1:160, whereas the other ten had lower titres (see Table 1). Antibodies to the diffuse type of the early antigen (EA(D)) were high in only one case (R.A.), the remaining patients having low EA(D) titres (see Table 1). Only one patient (R.B.) out of 11 had a measurable antibody titre (1:40) to the restricted (R) type of EA, frequent in African BL patients; but serum from R.A. could not be evaluated in this regard.

Table 1 shows that positive hybridisation results were obtained with EBV cRNA on two separate tumour specimens from one patient (R.A.). Multiple EBV genome copies were present, as in African BL biopsies[2]. Another case (R.B.) was

Table 1 Serum titres and nucleic acid hybridisation with EBV ^{32}P-cRNA*

			Serum titres		^{32}P cRNA bound per 10 μg	EBV genome equivalents	No. of
Source of DNA	VCA	EA(D)	EA(R)	EBNA	DNA (c.p.m.†)	per cell‡	determinations
Calf thymus					218	0	6
Raji					4,860	60	6
R.A. bone marrow, (50% tumour cells)	320	160	?	160	1,014	10	6
R.A. lymph node (solid pieces)					3,158	38	4
R.A. lymph node (cells in suspension)					4,550	56	3
R.B. ascites	160	10	40	160	340	~2	4
R.K. ascites	10	< 10	< 10	20	221	< 1	2
S.V. ascites	10	< 10	< 10	2	229	< 1	2
P.R. pleural fluid	40	< 10	< 10	40	211	< 1	2
S.L. ascites	20	< 10	< 10	10	196	< 1	2
D.T. leukocytes (20% tumour cells)	80	< 10	< 10	2	229	< 1	2
S.L. leukocytes (90% tumour cells)	20	< 10	< 10	40	237	< 1	2
K.K. pleural fluid	< 10	< 10	< 10	< 2	201	< 1	2
R.L. testes	20	< 10	< 10	20	239	< 1	2
E.W. ovary	20	< 10	< 10	40	223	< 1	2
L.D. ascites	40	< 10	< 10	10	220	< 1	2

*Initial specific activity 1.2×10^8 c.p.m. μg^{-1}.

†DNA content on each filter after the hybridisation was determined by the diphenylamine reaction and used to correct the data to 10 μg DNA filter. For further details on the hybridisation procedure see ref. 2.

‡Number of EBV genome equivalents per cell were calculated with the assumption that the molecular weight of EBV DNA is 10^8 and that of cellular DNA 4×10^{12}.

borderline positive, because the data obtained were close to the limit of sensitivity of the hybridisation technique. The ascites specimen from this patient (R.B.) was EBNA positive (20% positive cells). As noted above, R.A. and R.B. were serologically unique in the present material, in having elevated EBV antibody titres and, in the case of R.B., even a clear EA(R) tire. Material from the other 10 tumour tissues gave negative results, that is, they could not have contained one EBV genome equivalent or more per cell.

The bone marrow aspirate of the positive RA patient contained approximately 50% tumour cells, and thus harboured about 20 EBV genome equivalents per tumour cell. Solid lymph node pieces contained at least 38 EBV genome equivalents per cell, whereas tumour cells in suspension contained about 56 EBV genome equivalents per cell. This patient was a white, 16-yr-old male with no record of malarial infection or travels to Africa. His clinical course was unusual in that he presented with cervical lymph node and bone marrow involvement several months after a syndrome compatible with acute infectious mononucleosis. Patient R.B. presented with large abdominal tumour and ascites; he died during induction therapy of acute pulmonary embolus.

Thus, in agreement with the results of Pagano *et al.*[4] the majority of American patients with the diagnosis BL lack the serological or viral genome association with EBV documented in African BL biopsies. One clearly positive exception has been identified in this study and one probable (borderline) positive. In addition, a BL biopsy from Israel has been found to be EBNA positive and EBV DNA-positive (Goldblum *et al.*, unpublished). Similar data have been obtained independently in other laboratories. Bornkamm *et al.*[5] have detected EBV DNA in a biopsy from a 6-yr-old German girl and Gravell *et al.*[6] and Epstein *et al.*[7] have each reported one case of an EBV DNA-carrying American BL patient. Thus, there is at present a total of five or six cases of non-endemic BL which harbour EBV in the tumour. The relatively large series reported here would suggest a rate of approximately 8–17% EBV-associated BL[8] in the non-endemic material. Although the histopathology of the non-EBV-associated American BL cases resembles typical African BL, it is not clear whether these non-endemic cases represent the same disease entity or should be classified differently from a clinical or aetiological standpoint.

In the high endemic regions of Africa, BL is known to arise from a single EBV-carrying cell in more than 95% of cases[9,13]. EBV-negative lymphomas classified as BL do not readily become infected with EBV *in vivo* even in cases in which the patients have been demonstrated to be carriers of non-malignant EBV-transformed cells. One patient (K.K.) in the present series developed classical infectious mononucleosis while in remission; nevertheless, tumour cells collected at the time of EBV seroconversion (VCA 1:40) were EBV genome negative. Horizontal infection *in vivo* may be hampered by the presence of virus-neutralising antibodies, sensitised memory T cells, and/or the virus receptor status of the tumour. The latter varies from total resistance to *in vitro* infection, to relative susceptibility in cultured EBV-negative BL lines[10-12].

Since histology and cytology are the sole criteria for the diagnosis of BL at present, the high frequency of EBV-positive tumours in endemic regions, and their rare occurrence in non-endemic areas, may imply disease heterogeneity in spite of morphological uniformity. It has recently been proposed that EBV-associated BL and EBV-negative BL may be two different diseases with very similar histopathological features[8], and there are abundant parallel examples of this in the experimental literature on known virus-induced tumours. If EBV is aetiologically important in EBV-associated BL, it would seem likely that one or more cofactors are involved which could be mediated by environmental (for example, malaria), genetic (for example, chromosomal), immunological (for example, impaired surveillance), viral or other mechanisms which exert a major influence in endemic regions, but a sporadic effect in non-endemic areas. This influence may not be identical in nature, but similar in action in these differing regions.

The Stockholm part of this work was carried out with the support of the Virus Cancer Program of the National Cancer Institute and of the King Gustaf V Jubilee Fund.

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Inhibition of the transition of a 40S ribosome–Met-tRNA$_i^{Met}$ complex to an 80S ribosome–Met-tRNA$_i^{Met}$ complex by 7-methylguanosine-5′-phosphate

7-METHYLGUANOSINE-5′-PHOSPHATE ($m^7G^{5'}p$) inhibits *in vitro* amino acid incorporation catalysed by several eukaryotic mRNAs[1]. The analogues, 7-methylguanosine-2(3)′-phosphate and unmethylated guanosine-5′-phosphate have no effect on these reactions, suggesting that the specific inhibition by $m^7G^{5'}p$ is related to the $m^7G^{5'}ppp$ cap known to be present at the 5′ end of many eukaryotic and viral mRNAs[2–12]. As a more direct test of this idea, we report here experiments with a fractionated protein chain initiating system from wheat germ in which mRNA is required for the conversion of a 40S–Met-tRNA$_i^{Met}$ complex to an 80S–Met-tRNA$_i^{Met}$ complex. Using several viral RNAs, we show that $m^7G^{5'}p$ inhibits only the mRNA-dependent step, and that this inhibition is demonstrable only with mRNAs that require a 5′ cap for translational activity.

The initiation process in the fractionated wheat system proceeds in three steps: formation of a factor–Met-tRNA$_i^{Met}$ complex, transfer of the complex to a 40S ribosomal subunit, and adjunction of the 60S ribosomal subunit to form an 80S ribosome–Met-rRNA$_i^{Met}$ complex. Figure 1*a* demonstrates the reaction leading to the formation of the 40S–Met-tRNA$_i^{Met}$ complex. This reaction requires two factors and GTP, and is unaffected by addition of mRNA. Figure 1*b* shows that further incubation at 3.6 mN Mg^{2+} with added ATP, mRNA and a third factor results in the formation of an 80S–Met-tRNA$_i^{Met}$ complex. The effect of several guanosine monophosphates on these reactions is examined in Table 1. Experiment 1 shows that the 40S reaction is unaffected in all cases. In the 80S reaction, $m^7G^{5'}p$ is strongly inhibitory, while the other guanosine mononucleotides have only a small effect (experiment 2), a specificity similar to that observed for the inhibition of overall amino acid incorporation[1]. The localisation of the inhibition by $m^7G^{5'}p$ to the reaction in which the 80S–Met-tRNA$_i^{Met}$ is formed, that is, the sequence in which mRNA participates, supports the idea that $m^7G^{5'}p$ inhibition is related to its structural analogy with the 5′-$m^7G^{5'}ppp$ present on most eukaryotic mRNAs.

As a further test of this conclusion, we studied the effect of $m^7G^{5'}p$ on the 40S–Met-tRNA$_i^{Met}$ to 80S–Met-tRNA$_i^{Met}$ reaction catalysed by RNA of satellite tobacco necrosis virus (STNV RNA). This RNA has been reported to have a 5′-terminal sequence of either ppApGpUp...[16] or pppApGpUp...[17] and we have further confirmed that it lacks an $m^7G^{5'}p$ terminus by examining the products of HIO_4 oxidation and ^3H-borohydride reduction (unpublished experiments with S. Muthukrishnan and A. Shatkin). We have also ascertained that STNV RNA does not require a $m^7G^{5'}ppp$ cap for its translation. Translation of STNV RNA is unaffected, either quantitatively (Table 2) or qualitatively (Fig. 2), by addition of *S*-adenosylhomocysteine (SAH), a methyl-transfer inhibitor known to prevent the 'capping' reaction[18]. Thus STNV RNA is ideal for testing whether the inhibitory action of $m^7G^{5'}p$

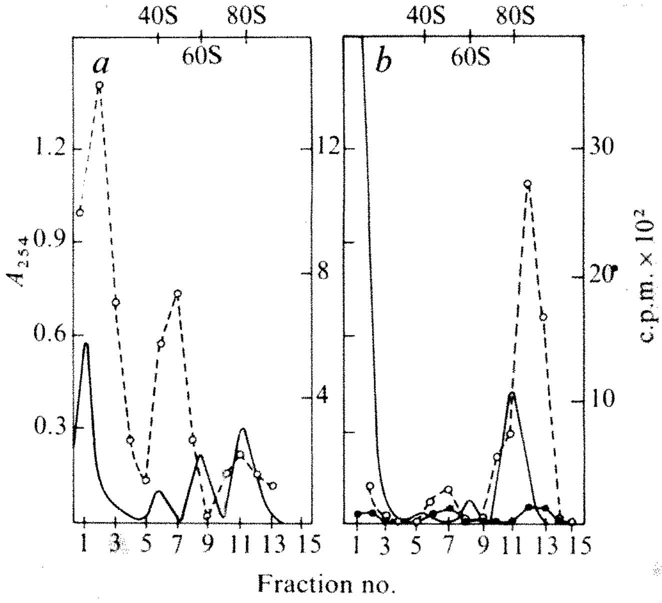

Fig. 1 Sequential formation of 40S–Met-tRNA$_i^{Met}$ and 80S–Met-tRNA$_i^{Met}$ ribosome complexes. *a*, 40S reaction. A reaction mixture (0.34 ml) containing 1 mM unlabelled Met; 30 mM Tris acetate, *p*H 8.0; 2.6 mM dithiothreitol; 60 μM GTP, 70 mM K acetate; 5 mM KCl; 1.3 mM Mg acetate; 7 mM phosphoenolpyruvate; 11.2 μg pyruvate kinase; 39 μg tRNA containing 6.6 pmol (CH_3–^3H)Met-tRNA$_i^{Met}$ (2,300 c.p.m. pmol^{-1}); 25 μg factor C3β; 150 μg factor D, and 115 μg ribosomes was incubated for 10 min at 20 °C. The samples were fixed with glutaraldehyde, centrifuged through a 13–21% sucrose gradient, and the fractions were analysed for bound aminoacyl-tRNA by nitrocellulose filtration. *b*, 80S reaction: the reaction was carried out in two steps. A reaction mixture identical to that of (*a*) was first incubated for 2 min at 20 °C, then 35 μg factor C3α, 6 μg AMV RNA, ATP (1 mM), Mg acetate (3.6 mM), and K acetate (70 mM) were added to a volume of 0.40 ml and the incubation was continued for 10 min at 20 °C. The samples were centrifuged without glutaraldehyde fixation through sucrose gradients and analysed as in (*a*). The open circles denote the data with the complete reaction mixture, while the solid circles denote data for a reaction mixture in which mRNA was omitted. AMV RNA is the RNA of the top "*a*" component of alfalfa mosaic virus[13] and was provided by Dr Lous Van Vloten-Doting. A complete description of the preparation of the wheat factors will be reported elsewhere.

Table 1 Effect of guanosine monophosphates on the formation of 40S–Met-tRNA$_i^{Met}$ and 80S–Met-tRNA$_i^{Met}$ complexes

	Addition	Met-tRNA$_i^{Met}$ bound (c.p.m.)	Inhibition (%)
(1) 40S reaction	—	1,701	
	$m^7G^{5'}p$	1,640	3.6
	$m^7G^{2(3)'}p$	1,829	
	$G^{5'}p$	1,876	
(2) 80S reaction (TMV RNA)	—	1,706	
	$m^7G^{5'}p$	342	80.0
	$m^7G^{2(3')}p$	1,468	14.0
	$G^{5'}p$	1,397	18.1
(3) 80S reaction (STNV RNA)	—	1,953	
	$m^7G^{5'}p$	2,147	
(4) 80S reaction (AMV RNA)	—	5,297	
	$m^7G^{5'}p$	1,363	74.3

The reaction systems were those of Fig. 1, with the viral RNAs added at 6 μg per 0.34 ml total volume. The data are a calculated sum of the nitrocellulose bound radioactivity; in experiment 1 for the region of the 40S complex and experiments 2–4 for the region of the 80S complex. The data in experiments 2–4 are corrected for a control in which mRNA was omitted. Procedures for preparation of TMV RNA and STNV RNA are described in refs 14 and 15, respectively. $m^7G^{5'}p$ and $G^{5'}p$ (PL Biochemicals) and $m^7G^{2(3')}p$ (TerraMarine Bioresearch) were added as indicated at a concentration of 0.25 mM.

is related to its structural similarity to the $m^7G^{5'}ppp$ 'cap' of an mRNA. If it were, one would predict that $m^7G^{5'}p$ would have little or no effect on the reaction catalysed by STNV RNA. Experiment 3 of Table 1 verifies this prediction. STNV RNA-catalysed conversion of the 40S–Met-$tRNA_i^{Met}$ complex to an 80S–Met-$tRNA_i^{Met}$ complex is unaffected by $m^7G^{5'}p$. Viewed in contrast to the results with tobacco mosaic virus (TMV RNA) (experiment 2) and alfalfa mosaic virus (AMV RNA) (experiment 4), both of which contain the 5′ 'cap'[10-12], the action of $m^7G^{5'}p$ indeed seems to be related to its structural analogy to the $m^7G^{5'}ppp$ 'cap' of mRNA.

The maximal inhibition that we have been able to obtain with either TMV RNA or AMV RNA is 80% even at $m^7G^{5'}p$ concentrations as high as 1 mM. With TMV RNA, it has been reported that only 70% of the viral RNA population contains the $m^7G^{5'}ppp$ 'cap'[10,11]. Thus the incomplete inhibition by $m^7G^{5'}p$ might be ascribed to residual activity of those RNAs lacking the 'cap'. We favour, however, an alternative explanation; that the translational requirement for the $m^7G^{5'}ppp$ 'cap' is not absolute even for those mRNAs containing the 5′ 'cap' but rather that the $m^7G^{5'}ppp$ 'cap' provides an additional binding site in the initiation system, thereby augmenting the translation of a particular mRNA. This possibility would allow that mRNAs might differ widely in the translational advantage gained by the 'cap', with the extreme situation being that exemplified by STNV RNA, where translation occurs readily in the absence of the 5′-$m^7G^{5'}ppp$.

This research was supported by grants from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania. We thank Dr Corrado Baglioni for making available his results before publication.

Table 2 ^{14}C-leucine incorporation catalysed by STNV RNA in presence and absence of SAH

Additions	^{14}C-leucine incorporated (c.p.m.)
—	1,200
STNV RNA (2.5 μg)	24,331
STNV RNA + SAM (4 μM)	26,752
STNV RNA + SAH (320 μM)	25,250
reovirus RNA (2.5 μg)	8,873
reovirus RNA + SAM (4 μM)	13,944
reovirus RNA + SAH (320 μM)	2,480

A 0.1-ml incubation mixture containing 20 mM HEPES-KOH, *p*H 7.6, 1 mM ATP, 20 μM GTP, 8 mM creatine phosphate, creatine phosphokinase (40 μg ml^{-1}), 2.5 mM dithiothreitol, 2 mM Mg acetate, 80 μM spermine, 20 mM KCl, 120 mM K acetate, 30 μM 19 amino acids–leucine, ^{14}C-L-leucine (0.625 μCi ml^{-1}), wheat tRNA (15 μg ml^{-1}), 25 μl wheat germ S23, and viral RNA as indicated, was incubated for 60 min at 25 °C. The hot TCA-insoluble radioactivity was determined as before[14]. Wheat germ S23 was prepared by a modification of the earlier procedure developed for wheat embryo[19]. A sample of 1.5 g of commercial wheat germ (General Mills) was ground in 10 ml 1 mM Mg acetate, 2 mM $CaCl_2$, 90 mM KCl using a chilled mortar and pestle. The homogenate was centrifuged at 23,000*g* for 10 min (pellet discarded), adjusted to 20 mM Tris acetate, *p*H 7.6, 2 mM Mg acetate and recentrifuged as above. The supernatant was then passed through a 25 × 1.5 cm column of Sephadex G-25 and eluted with 1 mM Tris acetate, *p*H 7.6, 50 mM KCl, 1 mMg acetate, 4 mM β-mercaptoethanol. The turbid fraction was collected, centrifuged at 23,000*g* for 10 min (pellet discarded), and stored in small samples at −70 °C. Reovirus RNA with a 5′-terminus of 75% ppGp and 25% GpppG was a gift of M. Morgan and A. Shatkin. *S*-adenosylmethionine (SAM) and *S*-adenosyl homocysteine (SAH) were obtained from Sigma.

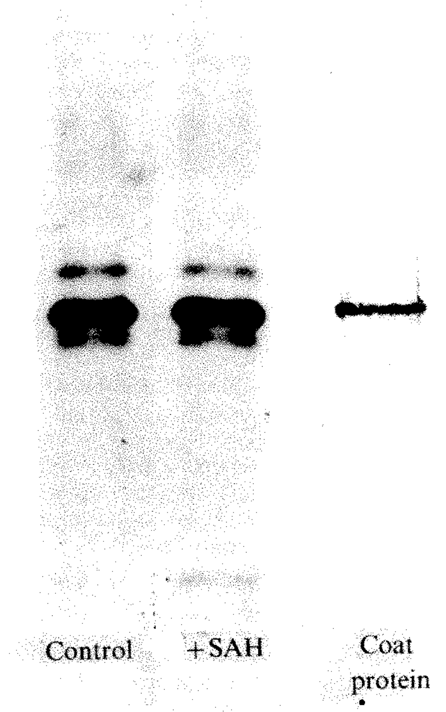

Fig. 2 *In vitro* products synthesised in response to STNV RNA. The *in vitro* incorporation reaction was carried out as in Table 2 except that a 19-methionine amino acid mixture and 20 μCi ^{35}S-L-methionine were used in place of leucine. The reaction was terminated by the addition of 10 mM Na_2-EDTA, *p*H 7.0, 10 mM L-methionine, and ribonuclease (0.1 mg ml^{-1}). After 15 min at 25 °C the mixture was cooled and precipitated with 9 volumes of cold acetone. The precipitated protein was air dried, dissolved in 4 M urea, 2.5 mM dithiothreitol, 1% sodium dodecyl sulphate, 50 mM Tris-HCl, *p*H 6.8, 1 mM Na_2-EDTA, *p*H 7.0, and heated at 100 °C for 2 min. Aliquots of the sample were applied to 15% polyacrylamide–urea–SDS slab gels prepared according to Studier[20], and electrophoresed at 80 V for 5–6 h. The gel was stained with 0.75% Coomassie blue, destained with H_2O–methanol–glacial acetic acid (7:4:1), dried and autoradiographed. Authentic STNV coat protein was prepared according to Lesnaw and Reichmann[21].

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Received December 22, 1975; accepted January 26, 1976.

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Structure of the lactose operator

THE *lac* operon has long been an important model system for developing an understanding of the control of gene expression. Since the early studies of Jacob and Monod[1] on the *lac* operon, much work has been devoted towards determining the mode of interaction between repressor and operator. Although it has been shown clearly that repressor physically binds operator[2,3],

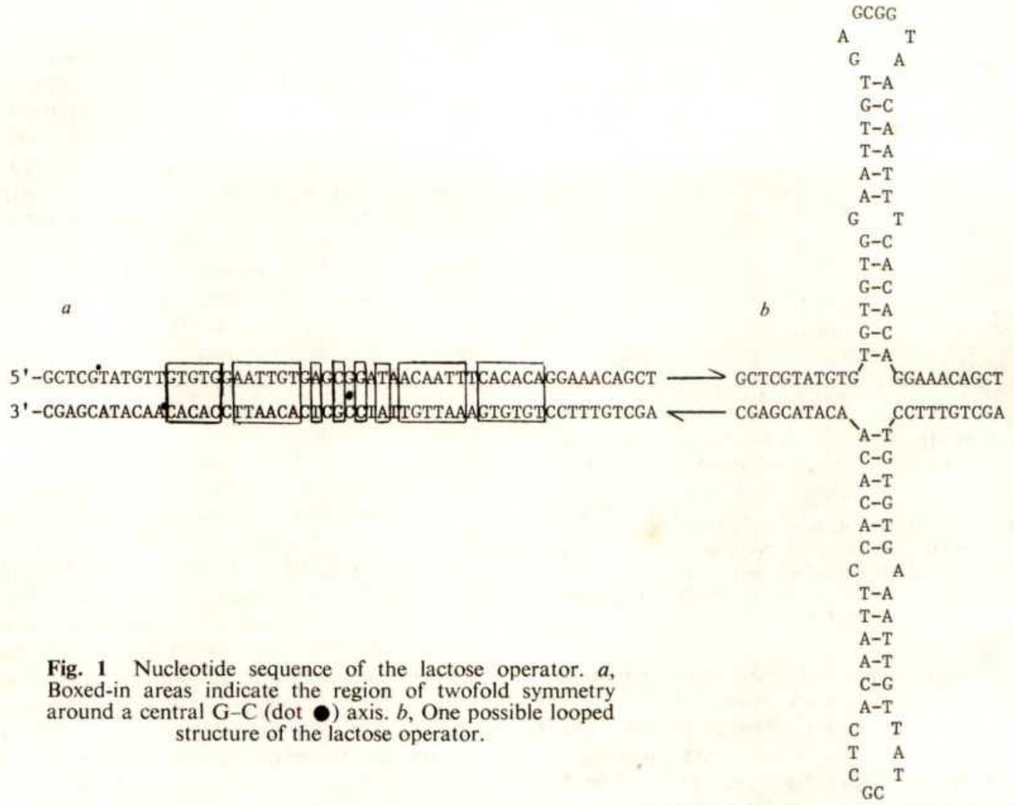


Fig. 1 Nucleotide sequence of the lactose operator. *a*, Boxed-in areas indicate the region of twofold symmetry around a central G–C (dot ●) axis. *b*, One possible looped structure of the lactose operator.

the details of this interaction remain unknown. We report here results of experiments designed to investigate further the structure of the operator and its interaction with the repressor.

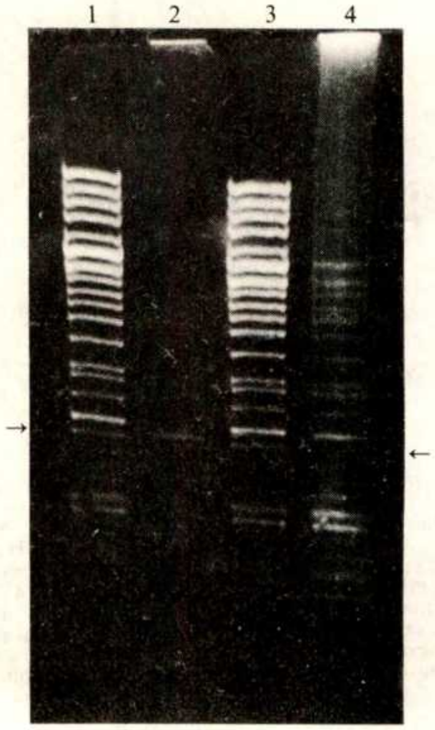
The interaction between *lac* operator and *lac* repressor is one of the most highly selective processes in nature. What is it about the *lac* operator, a sequence of approximately 25 nucleotides[4], which allows *lac* repressor to select it from among one million bases in *E. coli*? Basically, when considering the mode of interaction between repressor and operator, theories fall into two categories: those which call for a major alteration such as single-stranded loop formation of the operator DNA, and those which do not. It has been proposed that twofold symmetry would be important in DNA–protein interactions[5], that regulatory proteins would bind to and stabilise single-stranded loop structures in DNA[6], and that formation of a loop structure would allow a protein with two or four subunits to interact with symmetrical DNA sequences[7].

The determination of the nucleotide sequence of the *lac* operator[4,8] (Fig. 1) served to amplify all the above questions. The *lac* operator does show twofold symmetry, in fact the type of symmetry within the *lac* operator, termed a complementary palindrome[9], theoretically allows the *lac* operator to form a looped structure (Fig. 1). We have tried to determine whether such a looped structure is indeed present, by using single-strand specific nucleases as probes.

We have examined the actions of two single-strand specific nucleases, S1 and mung bean, on a DNA fragment containing part of the *lac* operon (see Fig. 2 for identification). The fragment, produced from λp*lac* 5 DNA by digestion with the *Hin*d restriction enzyme is approximately 700 base pairs long[10,11] and contains the carboxyl terminal sequence of the *i* gene, the entire *lac* control region and about 30% of the *z* gene. The *lac* operator region is located almost in the centre of the fragment.

Figure 3 shows the results of control experiments carried out by incubating heat-denatured ^{3}H-salmon sperm DNA with S1 nuclease (Fig. 3*a*) or mung bean nuclease (Fig. 3*b*). In 15 min, S1 nuclease at a concentration of only 0.1 U per μg DNA rendered 50% of the single-stranded DNA acid soluble, and mung bean nuclease at a concentration of only 0.0065 U per μg DNA rendered 80% acid soluble. Thus, at the much higher concentrations of S1 nuclease and mung bean nuclease used in

Fig. 2 λp*lac* 5 DNA (250 μg; prepared from strain G141 (λC$_1$857 λp*lac*5), a gift from Dr W. Gilbert, by the method of ref. 18) was digested with the *Hin*d restriction enzyme (prepared as in ref. 19 and a gift from Dr E. Jay). After phenol extraction and ethanol precipitation this digest was mixed with 1.5 μg of *lac* repressor (prepared as in ref. 20) and passed through a Millipore HAWP, 0.45-μm filter. The filter-bound material was eluted with 1 mM IPTG. All samples were precipitated in ethanol before application to a 20 cm × 20 cm × 0.3 cm slab gel of 1.4% Agarose (Seakem) and run in the presence of ethidium bromide. Electrophoresis was run at 80 V for 6 h. Slots 1 and 3 show the total *Hin*d digest of λp*lac*5 DNA. Slot 2 shows the fragment eluted from the filter with IPTG. Slot 4 shows the filtrate. The arrows indicate the region of interest. Note that in slot 2 there is only one fragment which is bound specifically by *lac* repressor and can be eluted from the filter by IPTG. This fragment is absent in the filtrate (slot 4).



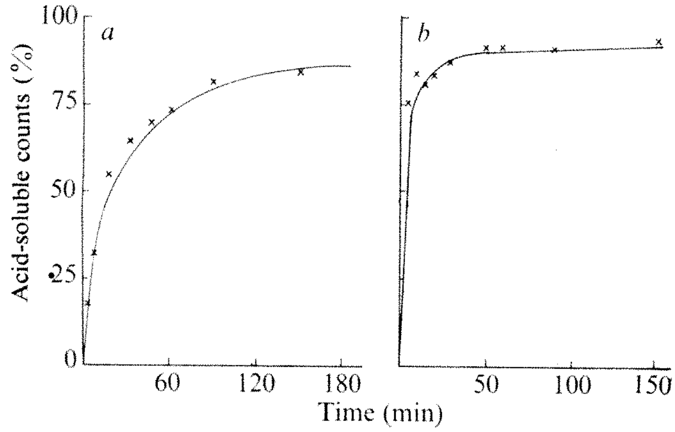


Fig. 3 Heat-denatured ^{3}H-salmon sperm DNA[21] was incubated with: *a*, S1 nuclease at 0.1 unit per μg DNA at 37 °C; and *b*, mung bean nuclease at 0.0065 unit per μg DNA at 37 °C. S1 nuclease was prepared by the procedure of Vogt[17] and was a gift of Dr G. Ghangas; the unit is as defined by Vogt[17]. Mung bean nuclease was a gift of Dr S. Laskowski, Sr, and the unit is as defined in Ardelt and Laskowski[22]. Incubations are as in the legend to Fig. 4. Each time point is the result of a triplicate determination.

the experiments below, single-stranded DNA would be hydrolysed to acid solubility in a few seconds.

The action of S1 and mung bean nuclease on the fragment is shown in Fig. 4. The incubation mixtures were analysed on 20×20×0.3 cm slab gels of either 1.4% Agarose or 4 or 5% acrylamide–7 M urea. Figure 4*a* shows an Agarose gel of *lac* DNA fragment after treatment with various concentrations of S1 nuclease. No specific cleavage of the fragment can be detected, even at high concentrations of the nuclease. Since *lac* operator lies almost in the centre of the *Hin*d-700 DNA fragment (arrow *A*), specific cleavage by S1 nuclease should yield two fragments about 350 base pairs long which would be clearly resolved (arrow *B*, expected location) from the starting material in the gel system. Analysis on acrylamide–7 M urea gels, which has a greater power of resolution, also failed to reveal any specific cleavage. Figure 4*b* shows the result of treating *Hin*d-700 *lac* DNA fragment with high concentrations of S1 nuclease as analysed on 4% acrylamide–7 M urea. S1 nuclease incubations were normally done at 50 mM NaCl, and varying the NaCl concentration at low S1 nuclease concentrations did not change the patterns shown. Results with mung bean nuclease were essentially identical to those with S1 nuclease. Even at the highest enzyme concentrations used, no specific cleavage of the fragment could be detected on either 1.4% Agarose (Fig. 4*a*) or 4% acrylamide–7 M urea gel (Fig. 4*b*). Fragment reisolated from the gels after nuclease treatment can still bind *lac* repressor with nearly the same efficiency as before. In some parallel experiments involving *lac* DNA fragment and S1 nuclease, a small amount of single-stranded ^{3}H-salmon sperm DNA was included in the reaction mixture. An aliquot was taken, after S1 nuclease treatment, to determine the extent of acid solubility. In all cases the single-stranded DNA was rendered at least 70% acid soluble.

Some streaking of the DNA band on gel and a reduction in the intensity of the starting material was observed when very high concentrations of the enzyme or low concentrations of salt were used. This may be due to either a nonspecific degradation of the *lac* DNA fragment by a contaminant nuclease or nonspecific degradation by S1 nuclease of the DNA fragment at areas where "breathing" occurs. That this degradation is nonspecific and not due to a single-stranded looped structure in the *lac* operator was demonstrated by treating a control DNA fragment that does not contain the *lac* operon with the same concentrations of S1 nuclease or mung bean nuclease. Gel patterns identical to those given in Fig. 4*b* were found.

If the *lac* operator existed as a single-stranded structure single-strand specific nucleases would be expected to cleave the *Hind* fragment carrying the *lac* operon into two pieces of about equal size. We did not find this to be the case and conclude, therefore, that in the absence of repressor, the *lac* operator does not contain single-stranded regions to any appreciable extent. The same conclusion was reached by Wang *et al.*[12], who measured the changes in superhelicity of *lac* DNA on binding of repressor, and also by Richmond and Steitz[13] who studied the binding of *lac* repressor to poly d(A)-d(T) fragments and suggested that the repressor binds to native DNA. On the other hand, Chan and Wells[14] have shown by competition binding assays that S1 nuclease- or mung bean nuclease-treated λp*lac*5 DNA loses the ability to bind *lac* repressor. This result implies

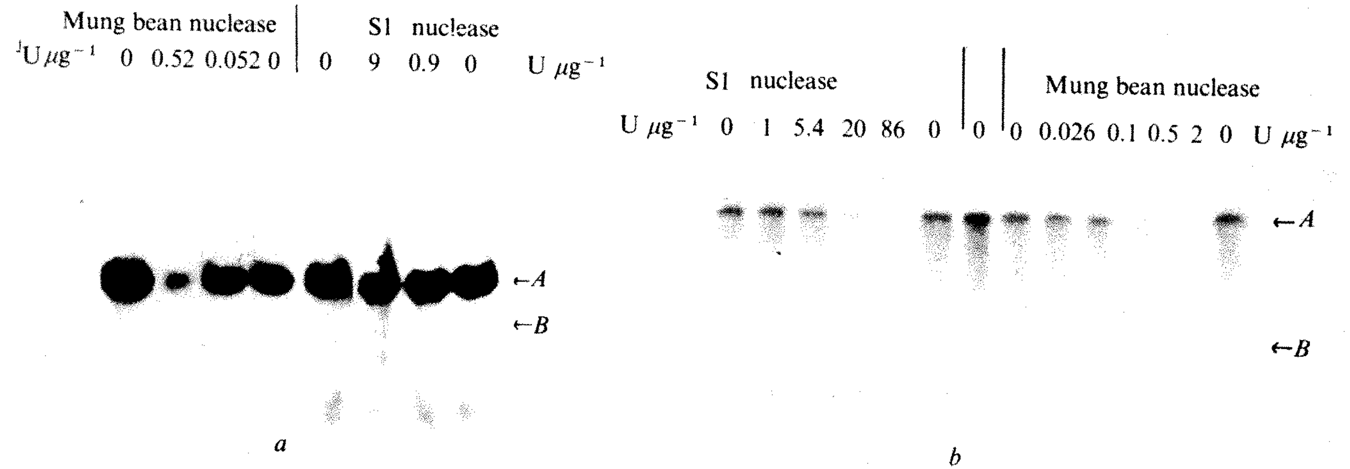


Fig. 4 *lac* fragment, identified as in Fig. 2 and purified as described[23], was labelled terminally at the 3′ end with α-^{32}P-GTP or CTP using terminal transferase as described by Kossel and Roychoudhury[24]. The fragment was then treated with a nuclease as follows. S1 nuclease: 30 mM NaOAc, *p*H 4.6, 1 mM $ZnSO_4$, 5% glycerol, NaCl varied between 10 and 150 mM in 100 μl. DNA concentration was from 1 to 10 μg ml^{-1}. Mung bean nuclease: 25 mM NaOAc, *p*H 5.0, 1 mM 2-mercaptoethanol, 10 μM $ZnSO_4$ and 0.001% Triton X-100 in 100 μl. DNA concentration was from 0.1 to 1 μg ml^{-1}. Digestions were stopped by the addition of EDTA to 25 mM. Protein was removed by phenol extraction (twice). The DNA was then ethanol precipitated. The samples were dissolved in 20 μl of loading dye (*a*, 10 mM Tris-Cl, *p*H 7.9, 1 mM EDTA, 10% sucrose, 0.2 mg ml^{-1} bromophenol blue; *b*, as in *a* plus 7 M urea). Electrophoresis is from top to bottom on 20×20×0.3 cm vertical slab gel apparatus. The wet gels were autoradiographed. *a*, *lac* fragment treated with S1 and mung bean nucleases at the indicated concentrations and then electrophoresed (80 V, 3.5 h) on 1.4% Agarose gel. *b*, *lac* fragment treated with S1 and mung bean nucleases at the indicated concentrations were boiled in loading dye plus 7 M urea for 5 min before electrophoresis on 4% acrylamide–7 M urea at 80 V for 24 h.

that S1 nuclease is cutting at or near the *lac* operator since binding of *lac* repressor before nuclease treatment abolishes the effect. Our experiments clearly demonstrate that a specific cleavage by a single-strand specific nuclease is not occurring within the *lac* operator region.

The nuclease concentrations used in our experiments are extremely high compared with those needed to hydrolyse single-stranded nucleic acid. Rushizky *et al.*[15], have shown that 0.0017 U of S1 nuclease per μg of MS2 RNA hydrolysed the RNA to acid solubility in 3 h at 23 °C. In addition, Shenk *et al.*[16] have cleaved linear SV40 DNA, nicked in only one position, into two intact and distinct double-stranded pieces with S1 nuclease at a concentration equivalent to 90 U (of Vogt[17]) per μg DNA at 25 °C in 30 min. They were also able to cleave DNA containing one mismatched base pair with the same concentration of nuclease.

We have been able to duplicate (qualitatively but not quantitatively) the results of Chan and Wells[14] in intact λp*lac*5 DNA using competition binding as an assay, however, the same experiments analysed by electrophoresis in 0.4% Agarose gel showed no specific cleavage of the DNA. We therefore conclude that Chan and Wells may be observing a secondary or indirect effect of the nuclease which, perhaps by nicking certain A–T-rich regions of the DNA, somehow alters the repressor binding of the *lac* operator region.

This work was supported by Grants from the National Institutes of Health and the National Science Foundation.

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Received December 1, 1975; accepted February 6, 1976.

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Cycling of RNA-directed DNA polymerase on natural and synthetic RNA templates

RNA-DIRECTED DNA polymerase (reverse transcriptase) catalyses *in vivo* the synthesis of DNA complementary to the RNA of tumour viruses. The DNA is integrated into the host genome and is therefore implicated in the subsequent tumorigenesis[1]. The mechanism by which reverse transcriptase replicates the viral RNA into complementary DNA (cDNA) and its resultant integration, as well as the interaction of the viral components in the host, remain unclear[2]. Reverse transcriptase has been used *in vitro* to synthesise cDNA to mRNA; this cDNA can be used to identify and characterise the cellular processes dependent on nucleic acids. The conditions of the reactions have been optimised[3,4]; however problems remain with both the quality and quantity of the cDNA[5,6,15]. We report here *in vitro* conditions such that each RNA-directed DNA polymerase molecule synthesises several to more than 100 transcripts, depending on whether natural or synthetic templates are used. As noted previously[3,4], the fidelity of the cDNA transcript, the amount, and the time course of polymerisation depend strongly on the reaction conditions.

Highly purified DNA polymerase from avian myeloblastosis virus (AMV) strain BAL was prepared by the method of Kacian and Spiegelman[7]. Purification included collection of the virus from plasma by high speed centrifugation, batch elution from DEAE–cellulose and chromatography on phosphocellulose. The enzyme was concentrated by dialysis against: 50% glycerol; 0.2 M KPO_4, *p*H 7.2; 2 mM dithiothreitol and 0.2% Triton X-100. This preparation (No. XL 13-75) contained 61 μg of protein per ml and had an activity of 2,476 U ml^{-1}. The protein concentration was determined by the method of Lowry.

Examination of this preparation of DNA polymerase by sodium dodecyl sulphate (SDS) polyacrylamide gels[8] demonstrated three distinct bands corresponding to the α and β subunits of the DNA polymerase and a contaminating band corresponding to a polypeptide chain of molecular weight 80,000. Based on the relative intensities of the stained bands the enzyme preparation was approximately 80% pure.

The reaction conditions are modified from those previously described[9] and are optimised for the reinitiation and elongation of the cDNA. The increased purity of the enzyme preparation facilitated the establishment of conditions for the maximum synthesis of cDNA per unit of enzyme (Table 1). The optimal

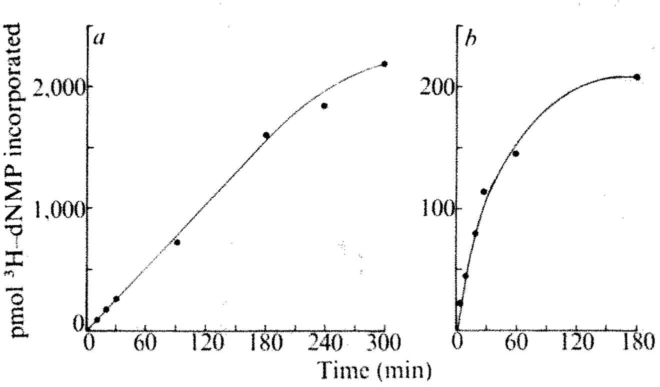

Fig. 1 Time course of cDNA synthesis as directed by poly(C) and rabbit reticulocyte globin mRNA. *a*, Time course of dGMP incorporation into cDNA directed by poly(C). The amounts of template, primer and radioactive nucleotide per ml were 570 μg of poly(C), 460 μg of $oligo(dG)_{10}$, and 25 μCi of 8-^{3}H(*N*)-deoxyguanosine 5′-triphosphate, specific activity 9.4 Ci $mmol^{-1}$ (New England Nuclear). *b*, Time course of dCMP polymerisation into cDNA directed by globin mRNA template. The concentrations of primer, template and radioactive nucleotide per ml were 54 μg of globin mRNA, 24 μg of $oligo(dT)_{10}$ and 25 μCi of 5-^{3}H-deoxycytosine 5′-triphosphate, specific activity 22.8 Ci $mmol^{-1}$ (New England Nuclear) or CH_3-^{3}H-deoxythymidine 5′-triphosphate (specific activity 49.8 Ci $mmol^{-1}$, New England Nuclear). Oligoguanylic acid and oligothymidilic acid primers were obtained from Collaborative Research; poly(C) template with an $S_{20,w}$ value of 3.08 was obtained from P.L. Biochemicals, and rabbit reticulocyte 10S mRNA was prepared as before[10]. Each 50 μl of reaction mixture contained the following components: 50 mM Tris-HCl, *p*H 7.9; 100 mM KCl, 10 mM $MgCl_2$; 8 mM dithiothreitol, actinomycin D (100 μg ml^{-1}) and AMV DNA polymerase (30 U ml^{-1}). The substrates were 320 μmol of each of the four deoxynucleoside triphosphates. The reaction mixtures were incubated at 32 °C for the times indicated. The reaction was terminated by the addition of ice-cold 10% trichloroacetic acid (TCA)–2.0% sodium pyrophosphate solution. After 30 min on ice, the TCA-precipitable material was collected by filtration on to prewashed (5% TCA–2.0% pyrophosphate) nitrocellulose filters. (Millipore type HA). The filters were then washed with 20 ml of TCA pyrophosphate, dried and the radioactivity determined by liquid scintillation spectroscopy.

Table 1 Effect of AMV DNA polymerase concentration on globin cDNA synthesis

Enzyme (pmol)	Globin mRNA (pmol) / Enzyme (pmol)	dCMP incorporated (pmol)	Product* (pmol)	Product (pmol) / Enzyme (pmol)
1.96	6.6	361	3.21	1.7
0.95	13	313	2.78	2.9
0.48	26	230	2.03	4.3
0.24	54	148	1.32	5.7
0.12	104	66	0.59	4.9

Reactions were run for 20 min as described in the legend to Fig. 1*b*.

* Calculation of cDNA synthesised is discussed in the text.

concentration of DNA polymerase was 0.24 pmol per 50 µl of reaction mixture. Although the turnover number (cDNA molecules synthesised per unit of enzyme per incubation) was maximised, the population of cDNA molecules synthesised was heterogeneous. We find that the ratio of DNA polymerase to template concentration affects both the quality and quantity of the cDNA polymerised. In the "slippage" reaction of the homopolymers (A) and (I), (which depend on multiple initiations) synthesis initiated with 400 ng of DNA polymerase synthesised an homogeneous product[9]; but polymerisation at 40 ng of enzyme results in a heterodisperse size distribution of the cDNA. This has also been observed for the globin mRNA-directed synthesis, although the shift in the size distribution was not as marked.

The ability to follow cDNA synthesis at prolonged time intervals (probably due to reduced RNAse activity in the enzyme preparation) is another improvement. The time course of globin mRNA-directed dCMP synthesis was compared with dGMP incorporation directed by poly(C) (Fig. 1). Incorporation differed in that the globin mRNA-directed synthesis of cDNA remained linear for only 20 min compared with more than 180 min for poly(C)-directed polymerisation. The homopolymer poly(A) or poly(I)-directed synthesis of a homopolymer cDNA was also linear for more than 180 min. The decrease in linearity observed with globin mRNA was not caused by template degradation as no stimulation was found when further mRNA was added (unpublished).

The comparison of the different cDNA products polymerised with globin mRNA, poly(C), poly(A) and poly(I) as templates is shown in Table 2. Poly(C)-directed incorporation of dGMP yielded 1,601 pmol and since the average template size was 55 nucleotides, 29 pmol of full sized product were formed. Dividing the moles of product formed by the moles of enzyme input, more than 100 cDNA molecules were formed per enzyme molecule. Repeating this calculation for globin mRNA as template the turnover number is 7, assuming the mRNA to be 650 nucleotides long[11] and composed of 17.3% GMP[12]. Synthesis directed by poly(A)$_{\overline{25}}$ or by poly(I)$_{\overline{35}}$ results in a product size larger than the starting material (16S to greater than 22S)[9]. The difficulty in estimating the ratio of enzyme molecules to product synthesised in a reaction which slips[13] (that is, the product is larger than the template) is that the product size is heterodisperse. Poly(A) and poly(I) templates directed the incorporation of 827 pmol of dTMP and 573 pmol of dCMP, respectively. Since these amounts are less than that incorporated into poly(C)-directed cDNA and since the products are much larger, the turnover numbers for the slippage reactions are small (≤ 1).

The calculation of turnover numbers assumes that the protein preparation is homogeneous and completely active. In this instance, a protein contaminate in the enzyme preparation was demonstrated. In the poly(C)-directed synthesis the primed portion of the cDNA is unlabelled and this may represent as much as 20% of the template molecule, and potentially more than 50% of the newly synthesised DNA. Finally, the product synthesised was assumed to be full sized. Although this seems to be true for the cDNA to poly(C) template (Fig. 2*a*), which

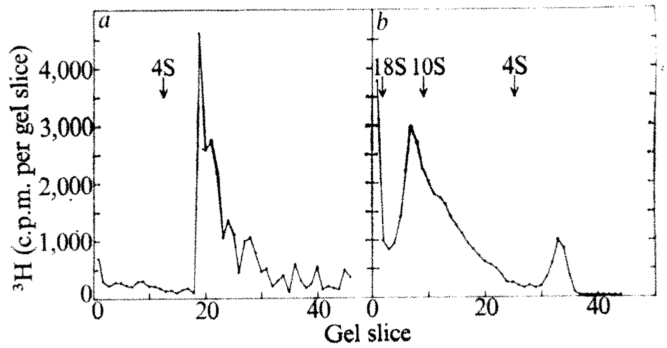

Fig. 2 Sizing of the cDNA product synthesised by DNA polymerase and directed by poly(C) and rabbit reticulocyte globin mRNA. Both reactions were carried out as described in the legend to Fig. 1 and incubated for 3 h. Poly(A) and poly(I) sizing was done as previously described[9] and is not shown here. The reaction mixtures used to obtain cDNA product for size analysis by polyacrylamide gel electrophoresis were stopped by heating to 100 °C for 2 min and then quickly frozen on dry ice. The RNA was hydrolysed with 0.33 N NaOH at 37 °C for 18 h. After neutralising the hydrolysate, unreacted nucleotides were removed by dialysis at 4 °C against 0.5 M NH_4HCO_3 and finally distilled water. The lyophilised samples were resuspended and a 20-µl sample was applied to 3.5% polyacrylamide gels containing sodium dodecylsulphate. The gels were standardised with rabbit reticulocyte 28S, 18S, 10S, and tRNA; poly(C), $S_{20,w}$ 3.08. *a*, Size distribution of the cDNA product directed by poly(C); *b*, size distribution of the cDNA directed by globin mRNA (as discussed in the text).

Table 2 Comparison of AMV DNA polymerase-directed synthesis with different templates

Template	Primer	Size of template	dNMP incorporated (pmol)	Product (pmol)	Molecules cDNA per enzyme molecule
Globin mRNA	$(dT)_{10}$	650	206*	1.8	7.2
Poly(C)	$(dG)_{10}$	55	1,606	29.2	115
Poly(A)	$(dT)_{10}$	25	827	?	?
Poly(I)	$(dC)_{10}$	35	573	?	?

Reactions were run for 3 h as described in the legend to Fig. 1. All calculations correspond to a 50-µl reaction volume. The size of the product was shown in Fig. 2*a* for the poly(C)-directed synthesis and Fig. 2*b* for the mRNA-directed synthesis. Slippage of poly(I) and (A) was described previously[9] and confirmed by sodium dodecyl sulphate (SDS)–acrylamide gels (standardised with MS2 RNA and φX174 DNA, Miles) as discussed in the legend to Fig. 2. Question marks indicate that product size was not calculated for these reactions because of the non-discrete product size, which occurs due to the slippage reaction with both poly(A) and poly(I) templates[9].

* The quantity of dCMP incorporated into rabbit globin cDNA. Calculation of cDNA synthesised to globin mRNA is discussed in the text

showed an electrophoretic mobility faster than the 4S marker and corresponds to the template size, the mobility of globin cDNA was considerably more heterogeneous (as demonstrated in Fig. 2*b*). As the poly(C) or globin mRNA-directed product was no larger than its template, the turnover numbers listed in Table 2 must represent minimal values.

The size distribution of globin mRNA-directed cDNA is broader than the homopolymer-directed cDNAs (Fig. 2*b*). This distribution may represent either intrinsic heterogeneity of the template as demonstrated by Vournakis *et al.*[14], or infidelity in the transcription reaction. No large (dT) homopolymers were detected and therefore there was no anomalous transcription of the poly(A) region as described by Falvey *et al.*[13].

In summary, RNA-directed DNA polymerase initiates many template primer complexes and transcribes full sized cDNA from a template of either globin mRNA or poly(C). *In vitro*, the enzyme is amenable to Michaelis–Menton analysis since a stoichiometric reaction has been ruled out. Whether this ability to utilise more than a single template applies *in vivo* or whether reverse transcriptase is intrinsically controlled to polymerise only a single viral molecule awaits future study.

L.J.K. was supported by a National Institutes of Health training grant at Johns Hopkins University School of Medicine. We thank Drs G. E. Houts, J. and D. Beard and the Office of Program Resources and Logistics, Viral Oncology, National Cancer Institute, for the RNA-directed DNA polymerase from AMV used in these studies.

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Polymorphism of a synthetic DNA in solution

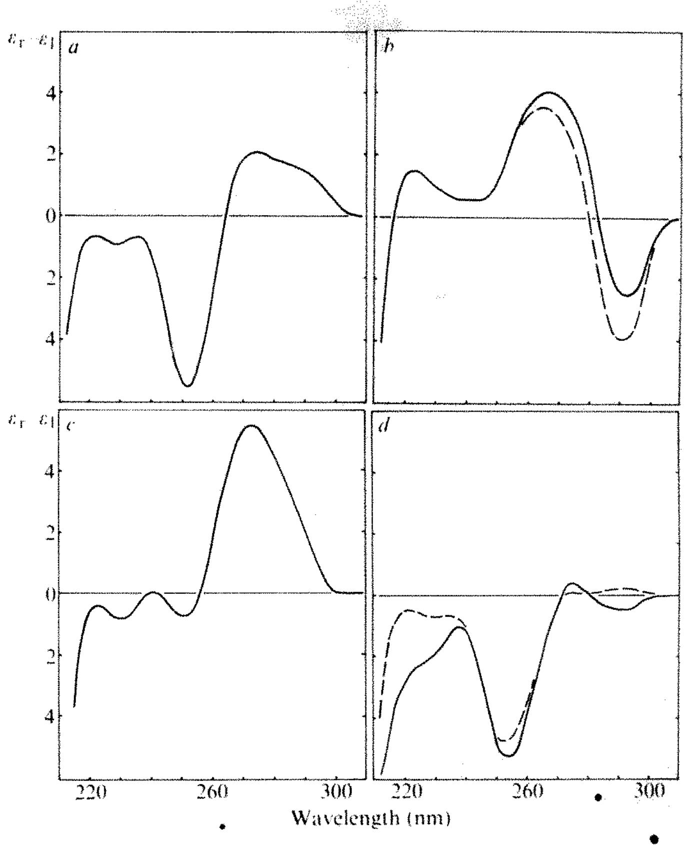
EARLY work on the X-ray diffraction of DNA fibres showed that DNA can adopt different conformations, and up to now at least four different double-helical models have been described in detail: the A, B, C, and D forms[1-4]. During recent years, it has become likely that DNA in solution also occurs in different conformations[5-7]. For studying such helix–helix transitions synthetic polynucleotides with a defined sequence offer an important tool, since in natural DNA only a very small fraction of the molecule usually has a particular sequence and, sequence-specific effects of the conformation will therefore escape detection.

We have previously shown that the alternating desoxypolynucleotide–poly(dG-dC)·poly(dG-dC) — undergoes a salt-induced, very cooperative helix–helix transition in neutral solution, which is most easily followed by the inversion of the circular-dichroism spectrum in the near ultraviolet[5]. X-ray diffraction studies by S. Arnott on fibres of the same sample of poly(dG-dC) have led to diffraction patterns characteristic for the D form and, at high relative humidity, for the B form, respectively[4,8] (personal communication). Since the fibres were pulled after ethanol precipitation of the polymers, it is possible that ethanol induces such a conformational change, which is then preserved in the fibres. I therefore measured the CD spectra of poly(dG-dC) as a function of the ethanol concentration. Figure 1 shows examples of CD spectra of poly (dG-dC)·poly(dG-dC) in different solvent conditions at room temperature. Between 0 and 45% ethanol the conservative spectrum observed is similar to that of natural DNA[9] (Fig. 1*a*). At 50% ethanol a drastic and fully reversible change of the CD spectrum occurs, giving rise to a conservative, but inverted spectrum (Fig. 1*b*). The absorption and CD spectrum, the high cooperativity of the transition and the relative slow kinetics closely resemble the observations at high NaCl concentration in the absence of ethanol[5]. It is very likely that both solvents induce a change to the same double helical form.

At still higher ethanol concentrations a more gradual change to a non-conservative spectrum (Fig. 1*c*), typical for double-helical RNA-like conformations is observed[5,6,10]. Figure 2 shows these changes of the optical properties as a function of the solvent composition. Thus, in ethanolic solution three distinct forms for poly(dG-dC) are observed.

A similar situation with three types of spectra exists in CsCl solutions in the absence of ethanol. Up to about 4 M CsCl the spectrum in Fig. 1*a* describes the data; around 4 M

Fig. 1 Circular dichroism spectra of poly(dG-dC)·poly(dG-dC) at different solvent composition. The polynucleotide was synthesised and characterised as described previously[5]. Ethanol was mixed with 1 mM Na phosphate buffer (*p*H 7.2) and a small volume of stock solution of poly(dG-dC) to a final concentration of 50–150 µM was added, and the CD spectra measured at 20 °C in a Cary 60 spectropolarimeter with CD attachment. Absorption spectra were measured with a Zeiss PMQ 3 photometer. *a*, 0-40% (w/w) ethanol or 10^{-3}–2 M NaCl. *b*, 56.2% ethanol; the broken line gives the spectrum in 3.9 M NaCl in the absence of ethanol[5]. *c*, 80 w/w % ethanol. *d*, Solid line, 7.1 M CsCl; broken line, 3.8 M LiCl.



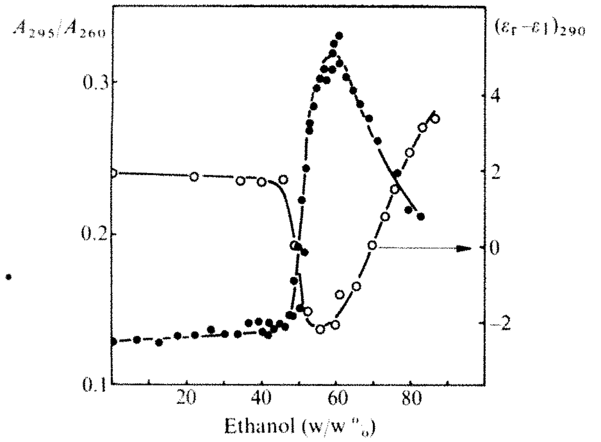


Fig. 2 Optical properties of $(\text{dG-dC})_{100}\cdot(\text{dG-dC})_{100}$ as a function of the ethanol concentration. Titrations were performed by adding 1 mM phosphate buffer or ethanol, respectively. Circular dichroism at 290 nm; absorbance at 295 nm divided by the one at 260 nm.

CsCl a cooperative transition to an inverted spectrum, like the one in Fig. 1*b*, is observed. At still higher CsCl concentration there is a gradual change to the spectrum shown in Fig. 1*d*, which is similar to the one obtained in LiCl solutions where no such inversion of the CD spectrum is observed.

The change of the solvent composition alone thus gives four different types of CD spectra for this polynucleotide and it is very likely that each one reflects a different double helical conformation. The type of spectrum in Fig. 1*a* together with the data on the Raman scattering of poly(dG-dC)[11] suggests that in "physiological" conditions the B form is adopted. At very high ethanol concentration the similarity of the CD spectrum (Fig. 1*c*) with the one for the corresponding RNA[5] is a strong indication for an A form of DNA, which is observed in fibres or films at very low water activity[12,13]. On the other hand, the non-conservative spectra at high CsCl or LiCl concentrations (Fig. 1*d*) closely resembles the one of Li-DNA in films[13] and may be attributed to the C form.

Assigning a known double-helical conformation to the inverted spectra of poly(dG-dC) in Fig. 1*b* leaves the D form as the most likely candidate. This hypothesis is supported by the Raman spectrum of this form, which does not show the features characteristic of the A, B or C conformation[11]. It also offers an explanation for the results of the X-ray diffraction studies with poly(dG-dC) mentioned above. For this type of diffraction pattern models for a right-handed[4] and a left-handed double helix[14] have been proposed. Although the CD spectrum is suggestive for the second alternative, no clear decision is possible, since calculations show that similar "inverted" CD-spectra can also be obtained for right-handed double helices (unpublished results). Although the assignment of these different double helical forms to the observed CD spectra has to be regarded as tentative, the experiments show nevertheless that relatively small changes of the medium around the DNA, as compared with the binding of proteins, allow a variety of conformational changes to occur in DNA in solution.

At the interface between two different helical conformations within a long DNA molecule one has to postulate for stereochemical reasons 'point defects' of 'dislocations' involving one or more base pairs. Such 'point defects' can also lead to 'kinks' within DNA molecules[15] facilitating the tight packing of DNA chromosomes or viruses.

I thank F. E. Freitag, the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for support.

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Received December 15, 1975; accepted January 30, 1976.

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Concentration quenching in chlorophyll

IN the primary process of plant photosynthesis it is generally accepted that efficient energy migration occurs between about 300 molecules of chlorophyll *a*, with subsequent light collection by a chemical trap. Solutions of chlorophyll *in vitro*, whether in fluid solvents[1], monolayers[2,3], multilayers[4,5] or, as shown recently in our laboratory, in rigid matrices of PMMA and in bilayer lipid vesicles, exhibit the phenomenon of concentration quenching of the excited state at concentrations much lower than those which are present in the chloroplast. At a chlorophyll concentration of 10^{-1} M, which is comparable with that in the chloroplast, none of the *in vitro* systems has a fluorescent yield as high as is found *in vivo*, especially when the photochemical traps are closed. To try to understand the apparent absence of concentration quenching *in vivo*, we have re-examined its mechanism *in vitro*, and conclude that each chlorophyll molecule in the light-collecting system must be separated from other chlorophyll molecules, so as to prevent trap formation by orbital overlap and so that the minimum distance, averaged over all orientations in a random array, is 10 Å.

A comprehensive review of the earlier work on concentration quenching has been given by Rabinowitch[6]. The fact that quenching occurs equally efficiently in media of high viscosity and in fluid solvents rules out diffusional processes and several authors have recognised that the concentration range of chlorophyll self quenching, in polar solvents such as ether and alcohols, indicates the involvement of Förster-type inductive resonance transfer between chlorophyll molecules[7,8]. There is no theoretical reason why this, in itself, should result in quenching but it could do so efficiently if a fraction of the molecules present acted as traps. This would be the case when non-fluorescent dimers are present and these undoubtedly account for most of the concentration quenching in such non-polar solvents as hydrocarbons and carbon tetrachloride, where the presence of the dimer is well established and characterised by a new absorption at longer wavelengths. We have made careful studies of absorption spectra in polar solvents such as ethanol and find no evidence of dimer formation at concentrations where extensive quenching occurs, nor are there any concentration-dependent changes in the absorption spectra of the varied systems mentioned earlier. At the highest concentrations used, the dimers would have had to exceed 10% of the total chlorophyll if they were the only traps present.

We therefore propose that the mechanism of concentration quenching in chlorophyll solutions is energy transfer between chlorophyll molecules by inductive resonance, followed by capture at a non-fluorescent trap which, in polar solvents, is merely a pair of chlorophyll molecules the separation of which, although part of the equilibrium statistical distribution, is less than a critical distance R_t. When one of a pair of molecules within this separation is excited, it interacts to form an excimer (fluorescing in the infrared, if at all) or other excited complex, as has been shown to occur in some aromatic hydrocarbons, even in rigid matrices[9].

To test this mechanism we calculated the rate of a random

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walk of the excitation in a random three-dimensional array of molecules by a process in which the intermolecular transfer rate is proportional to R^{-6} (R being the intermolecular separation). The approximations which have to be made to obtain an explicit solution to this problem introduce uncertainties which are difficult to assess and we have therefore used a Monte Carlo method of calculation. By selection of pseudo-random numbers from a uniform distribution, 1,620 molecules are positioned among 27×10^6 coordinates in a regular three-dimensional lattice. This is equivalent to placing points in a cube consisting of 300 planes with separation D Å. Excitation is introduced by selecting one point at random. Rates are then calculated for all the possible processes available to the initially excited molecule, namely, fluorescence, intersystem crossing and resonance energy transfer to all molecules in a sphere of radius $35D$ Å around the excited molecule. The energy is then transferred to a new molecule and the process is repeated until it is terminated by fluorescence, intersystem crossing or trapping by a pair of molecules closer than a distance R_t. The whole random walk was then repeated using further selections of arrays and the results averaged. The complete process was repeated for a range of concentrations, the concentration being changed by varying D. The lattice is large enough for the probability of excitation reaching the edge of the lattice to be negligible.

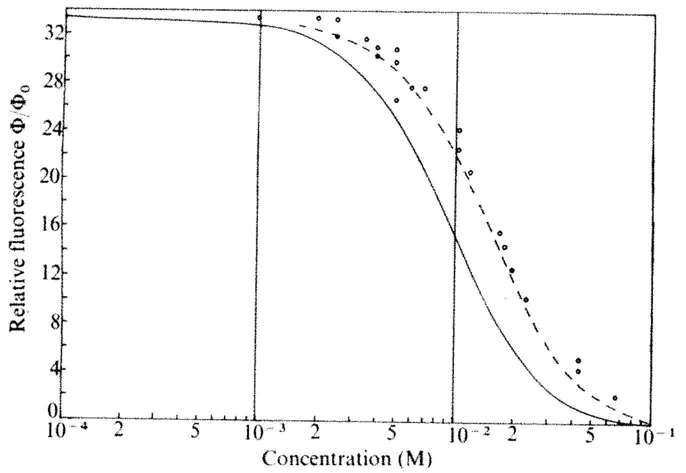

Fig. 1 Relative fluorescence yield of chlorophyll *a* as a function of concentration. ○, Experimental data of Watson and Livingston[1]; ———, calculated curve with $R_0 = 70$ Å and trap = 14 Å; - - -, calculated with $R_0 = 70$ Å and trap = 10 Å.

Complete fluorescence quenching curves for chlorophyll *a*, calculated in this way, are shown in Fig. 1. A separation of the statistical-pair trap was chosen for the calculation of each curve and the process stopped whenever the excitation reached such a pair. In Fig. 1, we have compared our calculations with the classical results of Watson and Livingston[1] for chlorophyll self quenching in ether. The shape of the curve of relative quantum yield against concentration is well reproduced and a quantitative fit is obtained for a statistical-pair trap separation of 10 Å. This may be compared with the diameter of the porphyrin ring in chlorophyll which is 15 Å. In these calculations, the Förster transfer distance R_0 was taken as 70 Å, the fluorescence and intersystem crossing rates as 5×10^7 s^{-1} and 1×10^8 s^{-1} which are appropriate for chlorophyll *a*. R_0 was obtained from the overlap of the measured absorption and fluorescence spectra in ethanol using the formulae given by Förster (see ref. 10). Obviously, the average distance of 10 Å between centres in the trap is so small that the orientation will become important. Our purpose here is merely to show how concentration quenching can occur, even in rigid media, when no dimers in the ground state are present. If dimers or oligomers are present, concentration quenching will be expected at even lower concentrations.

We now return to the mechanism of light collection by chlorophyll molecules in the photosynthetic unit. At a chlorophyll concentration in the chloroplast of 10^{-1} M, the fraction of molecules in statistical-pair traps with separation 10 Å would be 0.22 (if they were in random distribution) and efficient photosynthesis would be impossible. Transfer to a chemical trap would have to occur rapidly enough to compete efficiently with trapping by statistical pairs, which is not consistent with the rather high fluorescence yield of the antennae chlorophyll and the correspondingly long fluorescence lifetimes of ~100 ps, or even more when the chemical traps are closed[11].

Two molecules, which are within the statistical-pair quenching distance, may be prevented from quenching if they are separated by another molecule. Infrared, X-ray diffraction and other structural studies have shown[12,13] that chlorophyll molecules, whatever their environment, tend to coordinate the Mg atom, either to another chlorophyll or to a solvent molecule, so that chlorophyll with only four bonds coordinated to Mg probably rarely exists. The dimers and oligomers, which Katz suggests[12] comprise the antennae chlorophylls of the chloroplast, are almost non-fluorescent and strongly quenching. We therefore conclude that chlorophyll molecules in the chloroplast antennae, although at close distance, are separated from each other by strongly coordinated molecules which prevent collapse to an excimer or other quenching configuration. It is not yet certain whether antennae chlorophylls are associated with protein, with lipid or with the lipid–protein interface[14]. Galactolipids are present in a concentration two or more times that of the chlorophyll[15] and models show that the galactose part of the lipid coordinates well with the Mg of the chlorophyll within the lipid layer, which could thus ensure isolation of each chlorophyll molecule from other chlorophyll molecules. S. E. Carlin (unpublished results), has shown that when lecithin, in lecithin–chlorophyll vesicles, is replaced by galactolipids, the chlorophyll concentration required to give half quenching is approximately doubled, an effect which, although too small to explain the efficiency of the chloroplast, suggests that further work on these lines may be profitable.

Since this work was completed, Fenna and Matthews[16] have reported the first structure determination of a chlorophyll-containing protein, in which they find an irregular arrangement of the rings of the seven chlorophyll molecules, an average nearest-neighbour separation between centres of 12 Å and no evidence of interaction between them. Although this is a bacteriochlorophyll–protein, which is not part of the antennae system, its structure is admirably designed to promote energy transfer while inhibiting concentration quenching.

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Morphological transformation and chromosome aberrations produced by two hair dye components

A POSSIBLE carcinogenic hazard from hair dyes has been suggested recently by mutagenicity studies using the *Salmonella* tester strains developed in Ames' laboratory[1,2]. We had previously found a good correlation between mutagenicity produced by specific cigarette smoke condensate fractions in the *Salmonella* mutagenesis system[3] and oncogenic transformation in the mouse C3H/10T½CL8 cell line[4] developed in Heidelberger's laboratory. I have therefore studied transformation in this cell line after exposure to two hair-dye components, 2-nitro-*p*-phenylenediamine (2-NPPD) and 4-nitro-*o*-phenylenediamine (4-NOPD), both of which had been found to be highly mutagenic in the tester strain TA1538. Although 2-NPPD has greater mutagenic capacity in the presence of a microsomal activation system[1], I thought that 2-NPPD might be able to transform C3H/10T½CL8 cells since there is sufficient microsomal activity to convert certain carcinogens such as polycyclic hydrocarbons to their active form(s)[5]. Both 2-NPPD and 4-NOPD were found to produce significant morphological transformation in the C3H/10T½CL8 cell line. In addition, in view of the reported correlation between specific chromosome changes and the expression of malignant transformation[7,8] and the fact that known carcinogens rapidly produce chromosome aberrations[9], I sought and found chromosome changes after treatment with these hair dye components.

2-NPPD and 4-NOPD, provided by Dr Bruce Ames, were dissolved in dimethyl sulphoxide (DMSO) and incubated at various concentrations with the C3H/10T½CL8 for 24 h as described before[4,6]. Approximately 1 month later the dishes were stained and scored for the presence of transformed foci. Only type III morphologically transformed foci were scored since these produced sarcomas in most cases when isolated and injected into syngeneic C3H mice immunosuppressed by irradiation or rabbit anti-mouse thymocyte serum[6]. The results of two experiments are shown in Table 1. Morphological transformation was found in each experiment after treatment with 2-NPPD in doses from 10^{-3} M to 10^{-5} M, with an increase in transformation frequency at higher concentrations. Morphological transformation was also produced by 4-NOPD in the same range of doses. A concentration of 10^{-6} M 4-NOPD produced no morphological transformation. Fifteen transformed foci were observed in the dishes treated with 2-NPPD and 4-NOPD, whereas no transformed foci were seen in the control dishes treated only with DMSO. Only one type III focus has been found in DMSO-treated control dishes during the 2.5 yr that the C3H/10T½CL8 cells have been used in our laboratory for transformation studies. Transformation was also observed at non-cytotoxic doses of 2-NPPD and 4-NOPD, which further supports the fact that the two hair dye components induce morphological transformation rather than select for it.

Table 1 Morphological transformation produced by 2-NPPD and 4-NOPD

Agent	Dose (M)	Average PE (%)	Total type III foci/total dishes	Transformation frequency (%)
Control (DMSO treated)		15	0/61	<0.01
2-NPPD	10^{-3}	5	3/21	0.29
	10^{-4}	12	2/22	0.08
	10^{-5}	14	1/21	0.03
4-NOPD	10^{-3}	7	2/26	0.07
	10^{-4}	10	2/20	0.10
	10^{-5}	15	5/30	0.11
	10^{-6}	15	0/10	<0.07

Cells were seeded from log-phase mass cultures into 60-mm dishes (1,000 cells per dish) and treated 24 h later with the indicated doses of 2-NPPD or 4-NOPD. Exposure was for 24 h, after which the cultures were washed and maintained for 4–6 weeks. Type III morphologically transformed foci were subsequently scored in stained dishes as previously described[4,6]. Cytotoxicity was determined in similarly treated cultures containing 200 cells and stained after approximately 10 d. The transformation frequency is the percentage of cells that gave type III transformed foci corrected for the plating efficiency (PE), number of colonies formed as a percentage of number of cells seeded.

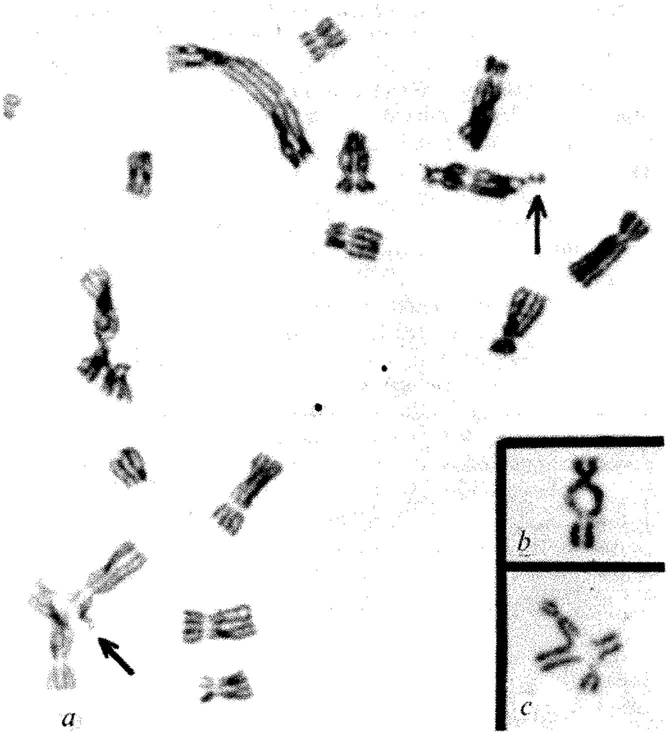

Fig. 1 *a*, Partial metaphase after treatment with 10^{-5} M 2-NPPD showing endoreduplication and two isochromatid fragments (arrows). *b*, An example of a dicentric chromosome seen after 2-NPPD exposure. *c*, A quadriradial chromosome configuration found after treatment with 2-NPPD.

The transformation frequency found with these hair dye components was approximately 10 times less than that produced by the potent carcinogenic polycyclic hydrocarbon, 3-methylcholanthrene, at equimolar concentrations[6]. This quantitative comparison, however, is made with caution for several reasons, including the fact that 2-NPPD, 4-NOPD and 3-methylcholanthrene represent different chemical classes and utilise dissimilar activation systems.

The hamster cell line, $A(T_1)Cl$-3, developed in our laboratory was used for the chromosome studies since this cell line contains a diploid number of hamster chromosomes[8] and is being developed as a rapid screen for chemical carcinogens[10]. The results are shown in Table 2. Both 2-NPPD and 4-NOPD produced significant chromatid breaks, with 2-NPPD yielding more chromatid breaks at the highest concentration tested. Several abnormal quadriradial and triradial chromosome formations as well as dicentric chromosomes were seen in metaphases after treatment with 2-NPPD (Fig. 1). The presence of dicentric chromosomes may indicate that 2-NPPD can be active in the G_1 phase of the cell cycle since this type of chromosome abnormality occurs after exposure to a chemical during G_1. Chromatid

Table 2 Chromosome breakage and other chromosomal aberrations produced by 2-NPPD and 4-NOPD

Agent	Dose (M)	No. of metaphases with indicated breaks per cell		Metaphases with indicated chromosome abnormalities
		0	1–4	
Control		50	0	None
2-NPPD	2×10^{-4}	30	20	1 Quadriradial, 3 triradials, 1 Dicentric
	10^{-4}	42	8	1 Quadriradial, 1 triradial, 2 Dicentrics
	10^{-5}	46	4	None
4-NOPD	3×10^{-4}	41	9	None
	10^{-4}	46	4	None
	10^{-5}	47	3	1 Ring chromosome

Hamster cells, A(T_1)Cl-3[8], were exposed for 24 h to various concentrations of 2-NPPD and 4-NOPD. One hour before collection colcemid (1 μg ml^{-1}) was added to arrest the cells in metaphase. The chromosome preparations were made as previously described and 50 metaphases for each time period were scored for the presence of chromatid breaks or gaps as well as other chromosome aberrations[11]. A gap was considered to be a lesion at least the width of a chromatid and a break was a gap with a different angle than the adjacent chromatid arm All lesions were reported as 'breaks' for simplicity.

breakage is indicative of chromosome damage produced in other phases of the cell cycle.

An endoreduplicated cell was found, after exposure to 10^{-5} M 2-NPPD, which contained two chromosome fragments (Fig. 1). This type of abnormal metaphase may be highly significant in the production of malignant transformation since at this non-toxic dose of 2-NPPD the surviving cell could easily be aneuploid, with chromosome imbalances. We believe such chromosome changes may be related to malignant transformation and oncogenesis[8,9]. The production of chromosome aberrations by 2-NPPD after exposure of human peripheral lymphocyte cultures has also been noted, although no chromosome abnormalities were found after treatment with 4-NOPD[2].

Considerable evidence now suggests that certain hair dye components could be potential hazards to humans, especially as these agents can be present in hair dye at concentrations as high as 10^{-2} M (ref. 11). The evidence includes their mutagenic activity in *Salmonella*, their ability to produce morphological transformation in the C3H/10T½CL8 cell line and their capacity to yield a significant number of chromosome breaks and rearrangements. Thus further studies of these and other hair dye components for carcinogenic and mutagenic potential *in vivo* seem advisable.

This work was supported by a grant from the US Public Health Service. I am a recipient of a career development award from the National Institutes of Health. I thank Anne Gardner, Mary Baker and Corey Mark for technical assistance. I also thank Drs Bruce Ames and Charles Heidelberger for critically reviewing the manuscript.

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Mechanism of selective nonspecific cell-mediated cytotoxicity of virus-infected cells

STUDIES of mechanisms of resistance to herpes simplex virus (HSV) in mice have demonstrated antibody-dependent cell-mediated cytotoxicity (AD-CMC) of HSV-infected cells exposed in suspension cultures to anti-HSV sera and normal mouse peritoneal cells (PC) or human peripheral blood leukocytes. In this system it was impossible to demonstrate T-cell-mediated cytotoxicity[1–3]. In an attempt to adjust the assay conditions to permit detection of such cytotoxicity by immune spleens or PC, the "monolayer" system of Gardner *et al.*[4] was examined in syngeneic, allogeneic and xenogeneic combinations. Target cell monolayers were infected with HSV and exposed to immune or normal mouse PC. The latter produced unexpectedly high levels of nonspecific release of ^{51}Cr by the monolayer HSV-infected cells. Because nonspecific or non-selective killing of human tumour cells[5] by lymphocytes from normal patients has been a major problem in human tumour immunology, we have investigated the mechanism of this nonspecific cell-mediated cytotoxicity in the HSV system. Our results indicate that selective nonspecific killing is a consequence of the cross linking of effector and target cells through aggregated immunoglobulins.

When HSV-infected cells, either Chang human liver cells or neuroblastoma C1300 clone N_2A cells of A strain mice[6], were exposed to normal uninduced mouse PC, either in "suspension" cultures as described before[1] or in monolayer cultures in which monolayers were infected for 18 h before addition of the PC, strikingly different patterns of cytotoxicity were observed (Table 1). While only low levels of ^{51}Cr release were found in suspension cultures of HSV-infected cells, quite high levels of cytotoxicity were found in monolayer cultures of HSV-infected cells and normal PC in a ratio of 1 : 20. This was a selective non-immune cytotoxicity and not an artefact of the culture conditions, for no such cytotoxicity was produced in identical cultures containing uninfected target cells (Table 1), or target cells infected with measles virus (unpublished observation). Addition of rabbit anti-HSV sera, as reported previously, generated a marked increase of ^{51}Cr release in suspension cultures attributed to an AD-CMC. In contrast, antibody-inhibited cytotoxicity in the monolayer cultures. The effector cells in this system were almost totally removed by passage over nylon wool columns[7] and were destroyed by preincubation with silica, known to be cytotoxic for macrophages[8]. Thus the nonspecific cytotoxicity in the monolayer system seems to be related selectively to HSV infection and is probably produced by macrophages.

It is known that infection of various non-lymphoid cells with HSV induces the expression of a receptor for the Fc region of immunoglobulins on the cell surface[9,10], and this was confirmed on our target cells. These observations suggested that the selective, nonspecific cytotoxicity observed in the HSV system might be mediated by cross linking of effector cells and target cells, both displaying Fc receptors, by aggregated immunoglobulins or immune complexes. Since most commercial foetal calf sera (FCS) contain some immunoglobulins, the monolayer assay was

Table 1 Cytotoxicity of peritoneal cells for HSV-infected Chang cells in monolayer or suspension cultures

Cells	Serum	% Specific cytotoxicity* HSV-infected Chang cells Monolayer	% Specific cytotoxicity* HSV-infected Chang cells Suspension	Uninfected Chang cells Monolayer
PC	—	65±6.3†	5±3.1	8±1.8
PC	Normal rabbit (1/100)	60±6.0	0±3.8	NT
PC	Rabbit anti-HSV (1/100)	41±5.3	89±8.3	12±3.5
—	Rabbit anti-HSV (1/100)	10±1.5	8±1.2	3±1.3

For the monolayer assay Chang human liver cells were dispensed in 1 ml of MEM + 10% FCS into flat bottom wells of multi-dish tissue culture trays (Linbro FB-16-24-TC) at a density of 1 × 10^5 per well. Three hours later the cells were infected with the HFEM strain of Herpes simplex (HSV) at an input multiplicity of 10. Twenty-four hours later each well was labelled with 2 μCi of $^{51}CrO_4$ (Amersham) for 1 h at 37 °C. Cells were washed three times. Uninfected cultures handled similarly served as controls. The suspension assay was carried out as before[1]. Uninjected normal rabbit serum or rabbit anti-HSV (neutralising titre of 1/800), was added to the monolayer or suspension cultures of target cells. PC were collected from C57BL/6 mice and were added at a ratio of 20:1 in a final volume of 1.0 ml. All assays were carried out in triplicate. Spontaneous leakage of ^{51}Cr was 15–20%.

*Percentage specific cytotoxicity was calculated according to the following formula:

$$\frac{\text{mean } ^{51}\text{Cr release in test group} - \text{mean spontaneous } ^{51}\text{Cr release}}{\text{mean } ^{51}\text{Cr release after freeze and thawing} - \text{mean spontaneous } ^{51}\text{Cr release}} \times 100.$$

* + s.e.m. in six to eight experiments.

carried out in medium containing certified agamma-FCS (Microbiological Associates, lot 87839). As Table 2 shows, the nonspecific cytotoxicity of HSV-infected murine neuroblastoma cells was markedly diminished in the agamma-FCS, and specific cytotoxicity could be seen. Introduction of rabbit immunoglobulins aggregated chemically by *biz*-diazotised benzidine[11] into the monolayer system significantly augmented the level of cytotoxicity in the agamma-FCS. This rabbit Ig had no detectable neutralising antibody against HSV. In parallel these aggregates blocked the formation of EA rosettes of sheep red blood (SRBC) cells coated with rabbit anti-SRBC antibodies[12] which detect Fc receptors on the effector cells. Nonspecific cytotoxicity-inducing activity of ordinary FCS could be diminished by ultracentrifugation at 100,000*g* for 1 h and use of the upper third of the serum (Table 2).

We believe that these results may have more general significance than just for the HSV system. Nonspecific cell-mediated cytotoxicity has been a serious problem in the interpretation of microcytotoxicity assays used to assess tumour immunity in man[5,13,14]. In this regard it is noteworthy that various primary tumours have been shown to exhibit Fc receptors[15]. Hersey *et al* reported nonspecific killing of melanoma lines by activated T lymphocytes possessing Fc receptors[14]. Similarly, complement receptor-bearing cells from blood of patients with infectious mononucleosis indiscriminately killed lymphoblastoid cell lines, whereas purified T cells had specificity only for target cells infected with Epstein–Barr virus[16] Stott *et al.*[17] found that bovine alveolar macrophages were cytotoxic for parainfluenza virus-infected targets but uninfected cells, and immune serum blocked this killing—findings similar to ours with the monolayer system. If this model were applicable to human tumour systems, nonspecific or nonselective cytotoxicity *in vitro* might similarly be mediated by linkage of effector and target cells through aggregated Ig or immune complexes. Cross linking through Fc receptors may be one mechanism of selective but nonspecific cytotoxicity, but it is unlikely to be the only one[18].

Table 2 Effect of aggregated Ig and FCS on Fc rosettes and cell-mediated cytotoxicity of HSV-infected neuroblastoma cells in monolayer cultures

Effector system (normal PC plus)	% ^{51}Cr release	% EA rosettes of P C
FCS	45	NT
FCS + anti-HSV serum (1/100)	11	NT
aγ−FCS	14	33
aγ+FCS+150 mg Agg, Ig	27	9
aγ+FCS+15 mg Agg, Ig	19	20
aγ+FCS+1.5 mg Agg, Ig	8	30
aγ+FCS+0.5% FCS	29	NT
FCS − after ultra-centrifugation (top third)	7	NT
(bottom third)	20	NT

Agg., aggregated

Monolayer ^{51}Cr cytoxicity tests were carried out as described in Table 1. Target cells were HSV-infected neuroblastoma cells. Ig aggregates (Agg.) were prepared described by Ishizaka *et al.*[11] by mixing rabbit Ig (10 mg ml^{-1}; Cohn fraction II, Pentex) with *bis*-diazotised benzidine (1/5 v/v) Inhibition of EA rosette formation on effector cells was tested as described by Westmorland *et al.* using rabbit anti-sheep red blood cells antibodies[8]. FCS was ultracentrifuged at 100,000*g* for 1 h.

Although it may be tempting to speculate that such a nonspecific cytotoxicity of cells exhibiting Fc receptors provides some level of non-immune surveillance or resistance to HSV infection or tumour growth, there is no evidence that this mechanism of cytotoxicity *in vitro* can provide protection *in vivo*.

This work was supported by grants from the US Public Health Service.

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matters arising

Maze-learning ability of *Drosophila melanogaster*

HAY[1] reported that the probability of right–left choices by *Drosophila melanogaster* in a uniformly illuminated multiple-step maze depends on the path through the maze before reaching the choice point. Animals which had initially turned right and were subsequently forced to take five right turns, by blocking the alternatives, had a greater tendency to turn right again than to turn left and vice versa. Hay interprets these findings to demonstrate that "*Drosophila* "learn" that this sequence of turns enables them to proceed through the maze towards the light" and "that the flies display associative learning. With no apparent positive or negative reinforcement they associate passage through the maze towards the light with a sequence of turns or, more accurately, with a particular orientation towards the light at each choice point, . . ."

In two-step maze experiments (designed for a different purpose) with *D. melanogaster* we observed similar deviations from binomial distribution[2]. These deviations were also found, however, in dark controls and, furthermore, when none of the alternatives in the maze was blocked such that the flies could proceed to the end of the maze by either route. We therefore do not agree with Hay's interpretation but would tend to relate these observations to the correcting behaviour or reverse turning observed from several different species[3–7].

To demonstrate our point of view we have performed several experiments which we report here. Figure 1 shows a scale drawing of the Plexiglas maze[8] used for these experiments. All dimensions are large compared with the size of *D melanogaster* except for the inner diameter of the funnels used to provide a one-way passage[2]. The collecting vessels were empty *Drosophila* culture flasks. The experimental setup was different from that of Murphey[9] in that our maze included forced turns between first and second choice points. The maze was washed and carefully cleaned before each run. Experiments were performed in complete darkness, at 22 ± 1 °C. Of approximately 400 flies (*Drosophila* wild type, Berlin, males plus females, age 3–10 d after eclosion) placed in the starting vessel usually 250 had completed the run after 12 h.

Table 1 shows the results of a typical experiment together with the total of 16 experiments. To eliminate the possibility that an inherent asymmetry of the maze causes the observed distribution, eight experiments were performed in orientation I (*a,b,c,d,*) and eight in orientation II (*b,a,d,c,*) by permuting the parts of the maze.

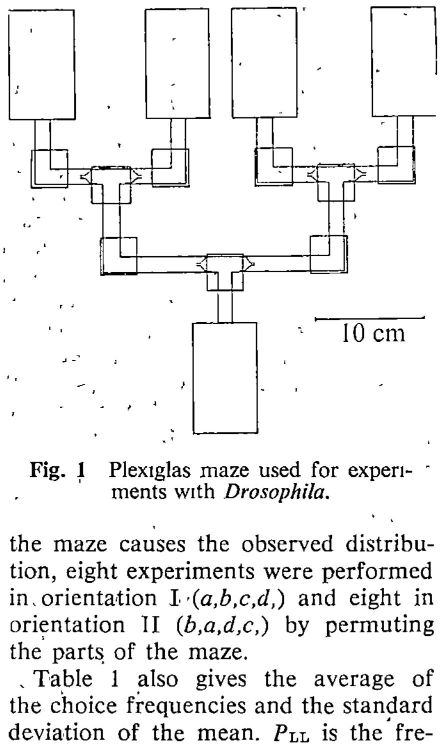

Fig. 1 Plexiglas maze used for experiments with *Drosophila.*

Table 1 also gives the average of the choice frequencies and the standard deviation of the mean. P_{LL} is the frequency of two subsequent left choices, P_{LR} is the frequency of a left choice and a subsequent right choice in the maze. It is obvious that $P_{LL} > P_{LR}$ and $P_{RL} < P_{RR}$. The differences are highly significant as tested with a *t*-test and a Wilcoxon rank sign test. The effect observed in our maze, however, is only about half as large as that reported by Hay[1].

Table 1 Performance of *Drosophila* in maze

	LL	LR	RL	RR
No. of flies counted in collecting vessels in a single experiment	75	45	39	69
Total of 16 experiments	1,331	903	903	1,391
Average of the choice frequencies	$P_{LL} = 0.296 \pm 0.019$	$P_{LR} = 0.202 \pm 0.012$	$P_{RL} = 0.196 \pm 0.015$	$P_{RR} = 0.306 \pm 0.015$
In Hay's notation	$P^*_{LL} = 0.602 \pm 0.021$			$P^*_{RR} = 0.604 \pm 0.021$

Hay reported an experiment in which all passages in the evenly illuminated maze were allowed and yet a nonbinomial distribution of flies was found. For our purposes we have only found it necessary to report that the effect is still observed in the dark. Thus it can hardly be associated with a successful "passage through the maze towards light". It is not intended to give an explanation[5] of this reverse turning behaviour; however, associate learning seems to be an unappropriate description.

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HAY REPLIES—I welcome the opportunity to expand the arguments in my report[1] on associative learning in *Drosophila.* There I listed and eliminated alternative explanations of the behaviour. Bicker and Spatz[2] have pointed out that one of these alternatives, namely correcting behaviour, could affect my results. Although I did not use the term 'correcting behaviour', it encompasses two of the variables I considered, namely centrifugal swing and spontaneous alternation.

The crucial experiment, however, (at the top of page 46 in my report), by which I distinguished correcting behaviour from associative learning is impossible in their case as they used only one strain of *Drosophila.* I demonstrated that, although there are strain differences in the probability of following the outer walls of a maze where all choices are available (which is what a correcting behaviour explanation would require), these strain differences are unrelated to the performance in the maze where choices are forced. That is, I accept that correcting behaviour is involved, but it cannot account for my strain differences in the latter maze in CL and CR (the changes between the first and second choice points in the probabilities of turning left or right) and it is necessary to introduce another variable, namely genetic differences in associative learning.

All Bicker and Spatz have done is to confirm Murphey's[3] demonstration that *Drosophila* show spontaneous alternation. This would explain why they obtained similar results in the dark and need not refute my suggestion that the flies associate passage through the maze with visual cues. In any case, one would expect very little learning in their maze, where there is only one forced turn between the two choice points, whereas my maze had a sequence of six forced right–left or left–right turns.

Finally, I should like to stress the need to consider both genetic variation and apparatus differences in maze learning work with *Drosophila*. First, I found large strain differences in learning, and other workers should similarly use a wide variety of strains, rather than just one, if they wish to demonstrate learning Second, Hay and Crossley (submitted for publication) showed with a maze rather like the one used by Bicker and Spatz but with an extra forced turn, that such apparently minor apparatus variations as the length of the connection between the start-tube and the first choice point can have very large effects on the probabilities of turning left or right. For example, the values of CL for males of the LM 20 strain varied from a mean ± s.e. of -0.069 ± 0.058 to $+0.491 \pm 0.102$ depending on the apparatus. Thus apparatus specifications need to be considered carefully in replicating the maze-learning work but in turn offer a means of studying in more detail some of the factors that determine turning preferences in *Drosophila*.

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Comments on a closed galaxy model for cosmic-ray propagation

Rasmussen and Peters[1] have presented a model of cosmic-ray propagation in which the particles do not escape from the galactic confinement region, but are eventually absorbed by meson-producing interactions with the interstellar gas. This contrasts with the generally accepted 'leaky-box' model in which cosmic rays escape from the Galaxy after traversing ~ 4 g cm^{-2} of interstellar gas. We consider here the intensities of positrons and γ rays implied by the two models.

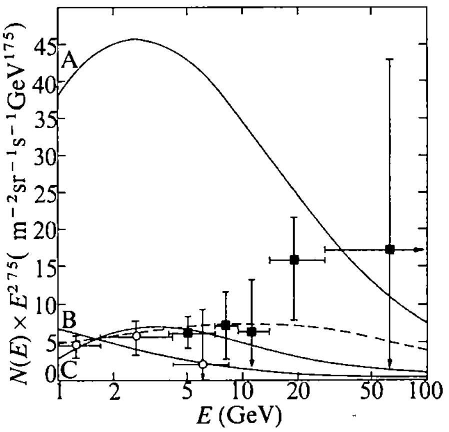

Fig. 1 Positron intensities above 1 GeV. — — —, Leaky-box model with average matter traversal of 4 g cm^{-2} and $E_{\mathrm{mod}} = 0.4$ GeV; ———, closed galaxy model with various values of E_{mod} and n_{H} (see text). ○, From ref. 3; ■, from ref. 4.

In Fig. 1, which is adapted from a figure of Orth and Buffington[2], the dashed line shows the positron intensity calculated by them for the leaky-box model. For the case of no escape of positrons, appropriate to the closed galaxy model, the solution of the diffusion equation for the positron intensity is

$$N(E) = q_0 E^{-\Gamma}(\Gamma-1)^{-1}(Eb+a)^{-1}$$

where $q_0 E^{-\Gamma}$ is the rate of production of positrons by the observed equilibrium nuclear flux. Their rate of energy loss is bE^2+aE, the first term corresponding to synchrotron and inverse Compton losses and the second to bremsstrahlung. Both q_0 and a are proportional to n_{H}, the effective mean density of interstellar gas traversed by the cosmic rays (nucleons cm^{-3}). Taking the rate of production of positrons given by Orth and Buffington; and $b = 10^{-16}$ GeV^{-1} s^{-1} the interstellar positron intensity is

$$N(E) = \frac{514E^{-2.65}n_{\mathrm{H}}}{(E+8.0n_{\mathrm{H}})}\,\mathrm{m}^{-2}\,\mathrm{sr}^{-1}\,\mathrm{s}^{-1}\,\mathrm{GeV}^{-1}$$

To compare this with observations, the modulation of the intensity by the interplanetary magnetic field has to be included. The modulation factor is expected to have the form

$$\exp(-E_{\mathrm{mod}}/E) \text{ for } E > 1 \text{ GeV}$$

The leaky-box spectrum in Fig. 1 was obtained using $E_{\mathrm{mod}} = 0.4$ GeV and $n_{\mathrm{H}} = 1$ cm^{-3}. Curve A is our calculated closed galaxy positron spectrum for the same values of these parameters. The observations show that either a lower n_{H} or a higher E_{mod} is required. It is not unreasonable to reduce n_{H} as a larger confinement volume is natural for the closed galaxy model. Curve B is obtained by fixing $E_{\mathrm{mod}} = 0.4$ GeV and varying n_{H} to give a weighted best fit to the observations. The optimum value is $n_{\mathrm{H}} = 0.027$ cm^{-3} implying that the lifetime of cosmic rays in the Galaxy is ~ 5×10^9 yr. If we regard E_{mod} as a free parameter we obtain the joint optimum values for n_{H} and E_{mod} of 0.1 cm^{-3} and 2.3 GeV respectively (curve C). It is not possible to rule out a modulation as large as this, at least down to 1 GeV, although the non-thermal radio background would favour the smaller value.

Independent evidence is provided, however, by the galactic γ-ray emission above 100 MeV from around the anticentre direction. Dodds *et al.*[5] have shown that if nuclear cosmic rays at their local interstellar density filled the disk out to the edge of the Galaxy, their interactions with the observed neutral hydrogen would produce twice as many γ rays as observed. Agreement with observation is obtained if, for instance, the cosmic-ray intensity decreases with galactic radius, R, in proportion to the mass density or if the intensity remains constant out to $R = 12$ kpc and is zero beyond. This type of behaviour is unlikely for the closed galaxy model, which favours a large confinement volume with a uniform cosmic-ray intensity. The γ rays are produced by cosmic-ray nuclei in the 1–10 GeV range. $E_{\mathrm{mod}} = 0.4$ GeV was assumed in deriving the local interstellar intensities of nuclei used by Dodds *et al.* from those observed at the Earth. For $E_{\mathrm{mod}} = 2.3$ GeV the intensity could be greater and the discrepancy in the γ-ray flux would be increased by a further factor of two. Thus we conclude that, although the closed galaxy model can be reconciled, as its authors state, with the observed positron flux, there are difficulties in making it agree at the same time with the observed γ-ray flux from the anticentre direction.

We thank A. W. Wolfendale and D. Dodds for helpful discussions.

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reviews

Tide turns for fisheries research

Fish Communities in Tropical Freshwaters. Their Distribution, Ecology and Evolution By R. H. Lowe-McConnell. Pp xvii+337. (Longman: London and New York, 1975.) £10.

To those accustomed to the restricted fish fauna of British freshwaters, the diversity in tropical freshwaters is bewildering. More than 8,000 of the 20,000 modern fish species live in tropical rivers and lakes; these include many suborders not represented in higher latitudes, for example, the electric fishes of the unrelated Gymnotoidei and Mormyroidei. The need to refer to many species which will be unfamiliar to most readers poses a problem to the author, who tackles it by providing a general chapter about the fish faunas of Africa, South America and South-east Asia, including five pages of excellent drawings of representative genera, by listing all the families in an Appendix and by giving a separate index of fish names, including synonyms.

Five chapters compare fish communities in the four main types of tropical freshwaters: equatorial forest rivers; seasonal rivers; natural lakes; and manmade lakes. All the lakes are African but Dr Lowe-McConnell is able to compare rivers in the three continents. For each locality, she outlines the topography and geological history, and then describes climate and resulting changes in water conditions. This treatment brings out clearly the similarities between physical and chemical conditions in rivers of the three continents and helps to explain the remarkable convergences in the adaptations of the fishes and the general structure of the communities in these waters. In contrast to temperate waters, there is usually a diurnal and a nocturnal fauna which never meet. The regular flooding of large rivers is associated with the habit of feeding and breeding in the shallow productive flooded areas and then facing the problem of retreating to the restricted river channels when the floods subside.

In three other chapters, Dr Lowe-McConnell makes a valuable contribution to current discussions about the origin and maintenance of diversity in tropical ecosystems by considering topics such as trophic relationships, breeding behaviour and speciation, especially in the African lakes. Her own wide experience in the tropics makes her comments vivid and penetrating.

This book should appeal to a variety of readers: it presents a comparative view of complex fish communities for all those interested in ecology and evolution; it provides a good basis for anyone starting detailed work on tropical fishes, either from an academic or from a practical point of view. The 20-page bibliography will be especially useful to fishery workers since it includes many papers which are relatively unknown because they are difficult to obtain. The only major complaint to make is the price—at £10, few students are likely to buy their own copies but it is a 'must' for teaching libraries as well as for many research workers. **Margaret Varley**

Fisheries Resources of the Sea and their Management. (Science and Engineering Policy Series.) By David Cushing. Pp. 87 (London: Oxford University, 1975.) £3 50.

THIS book is a brief but useful review of the world's fisheries to inform the uninformed. The first chapter deals with the recent history and development of the fisheries and fishing gear The world catch of fish amounts to 65–70 million tons, the predominant species being the anchovies, sardines and herrings, followed by the cod-like fishes, together amounting to 25 million tons.

Japan and the USSR have become the biggest fishing nations but fisheries research is practised in most maritime countries, its foundation being based mainly on work in the N.W. European countries. Dr Cushing gives a potted survey of age, growth, fecundity and migrations of fish, and the delimiting of stocks, with a longer chapter on fish population dynamics Following the earlier regulation of fisheries by mesh sizes of nets, minimum sizes of fish for landing or by close seasons, there has been a trend towards allocating quotas to countries. In spite of research and control, overfishing is still prevalent Nevertheless it is considered that up to 100 million tons could be taken annually, mainly by the exploitation of new stocks.

Dr Cushing surveys the history and practice of fisheries management and the various international councils and commissions. The international structure of management is extremely complex and these chapters are especially useful in giving a brief account of the efforts being made to control fisheries in a rational manner. Alas, national interests, fishermen's lobbies and disagreements between governments and scientists have meant that attempts at conservation have often ended in failure.

J. H. S. Blaxter

Principles of Fishery Science. By W. H. Everhart, A. W. Eipper and W. D. Youngs. Pp. 288. (Comstock: Ithaca, New York and London, September 1975.) £6.85.

Principles of Fishery Science is an updated abridged version of *Fishery Science: Its Methods and Applications* by Rounsefell and Everhart, first published in 1953 and aimed at graduate and upper division undergraduate students. In its original form this book soon became a classic at a time when fishery science was a rapidly expanding field. The authors have endeavoured to update the book in the light of enormous developments in fishery research during the past two decades. They stress, however, that the book is designed to present principles and applications of fishery management, and assumes that students and practising fishery biologists already have a foundation in the biological, physical and mathematical, and social sciences.

The first two chapters made me feel that the authors' endeavours had been successful. The Introduction refers to systems analysis and describes the new approach to research as a result of the general systems theory. The following chapter on Characteristics of Fisheries is also thought-provoking. The subsequent chapter on Fishing Gear, however, seems quite out of place. It interrupts the flow of the book and seems to have been lifted straight out of the 1953 edition.

Chapter 4 on Population Identification sets our train of thought back on the rails and brings us well up to date with the latest methods for separating populations using serology and X-ray fluorescence spectometry.

The following five chapters I am afraid contribute nothing new to the field. Many cheaper books (an important consideration for undergraduates), such as those produced for the International Biological Programme, deal with age and growth, population estimation, mortality, recruitment and yield in as much detail.

The authors come into their own when considering habitat improvement, pond management, screens and guiding devices and fish ways, subjects given considerable space in their earlier book. There is a slight imbalance at times (a result of three authors?), for example pollution is "written off" in four pages, although a two and a half page bibliography on the subject follows the chapter. Yet, under Control of Undesirable Species (Chapter 18), we have a 2-page description (pp263–265) of the new piscicide antimycin.

There are two useful chapters on the Role of Hatchery-reared Fish and Stocking, although the literature citations are practically all of work carried out in North America; this is a general criticism of the bibliographies at the end of all the chapters.

This is a useful working text for undergraduates, which I shall certainly suggest mine read. It is difficult, however, to recommend its purchase at its present price. I suggest that its contents be slightly reduced, perhaps by removing chapters 3, 9 and 11; and released as a paperback. It will then serve a very useful role. **Derek Mills**

Surface physics

Surface Physics. (Oxford Physics Series.) By M. Prutton. Pp viii+117. (Clarendon: Oxford; Oxford University: London, August 1975.) £2.75.

THE 'black magic' of surface effects is just starting to gell into science Physicists have seen good spectroscopic evidence of the 'dangling bonds' or 'surface states' of which they have talked for years; and now diffraction experiments can identify the reconstruction of the lattice which take place if a layer of atoms is deprived of its neighbours on one side. The concepts, although exciting, are still complex and novel; and thus any attempt to present the physics of surfaces to the undergraduate requires a controlled style, a very firm grip of concepts and will also gain tremendously from deep immersion in the prevailing currents of research. Dr Prutton qualifies in all these departments and has produced a fascinating and readable text, covering a surprising amount in an inexpensive 117 pages He also somehow manages to combine impartiality with an infectious enthusiasm.

For those solid-state device workers who may be preparing to rush precipitately to the bookshelves, it must quickly be said, however, that the systems covered here are all ultraclean vacuum-solid interfaces and the much more murky field of solid–solid interfaces will probably have to wait a long time before a similar burst of enlightenment manifests itself Ultrahigh vacuum techniques have made possible a battery of methods (LEED, XPS, UPS, ELS, AES and FEM, to name a few acronymically) which have revolutionised our knowledge of vacuum–solid interfaces but which cannot penetrate to internal interfaces. Even the device worker, however, can learn a great amount from this book about the detection of surface impurities and he can also begin to appreciate how the growth of thin films is so powerfully controlled by the surface forces described.

I particularly appreciated some clear explanations of Ewald sphere construction for two-dimensional 'nets' and the distinctions between ultraviolet and X-ray photoelectron spectroscopy. Most of the drawings are jewels of explanatory lore, the captions being nearly sufficient in themselves as a teaching instrument; the references to other texts are confined wherever possible to a few authoritative works.

Among the explanations which I found less than illuminating were those on field emission and the photoelectric effect Here, surely, are two properties well suited for demonstrating both the ideal electronic behaviour of surfaces and some direct practical uses of clean vacuum–solid interfaces as, for example, in photomultipliers. The great body of work on the effect of alkali atom adsorbates on the electron affinity of surfaces would have fitted rather well into the scheme of the book but is not mentioned. This is, perhaps the most clear example of a tendency in the book to emphasise structural features at the expense of electronic poperties; another example is the very brief treatment of the surface state as an electronic level, in spite of some classic studies recently published by Spicer, Eastman and others

With these reservations there is no doubt that this compact and inexpensive little book will engender the right kind of enthusiasm for and familiarity with surface physics in many universities and even sixth forms.

Andrew Holmes-Siedle

Mexikanischer Vulkane

Studien zur jungquartaren Glazialmorphologie mexikanischer Vulkane. (Mexiko-Projekt der deutschen Forschungsgemeinschaft, Vol. VII.) Pp 178. By Klaus Heine. (Franz Steiner: Wiesbaden, 1975.) Quarto, art. Hardback· DM82.00.

THIS work, based on an extensive field survey of the more accessible high volcanoes, sampling of sediments and mineralogical analyses, is extremely interdisciplinary. The geomorphological features are assigned to their relative sequence and absolute dates through the stratigraphical evidence afforded by intervening beds of pyroclastics and fossil soils. Both of these, being readily identifiable and very widespread in the region and often carrying at least limiting ^{14}C dates, enable correlations to be established between glacial features which are miles distant from each other, on more or less isolated volcanic piles up to 5,500 m in altitude

At no time, it seems, did even valley glaciers extend below the 2,500 m contour, whereas the three present-day, barely surviving ice caps all lie above 4,700 m. Five main glacier advances, marked by moraines, are recognised· MI, 38,000–32.000; MII, about 12,100; MIII, 1+2, about 10 000–9,000; MIV, c. 2,000; MV. 180-25 yr b p. These conclusions are supported by numerous sections distributed over a wide area described and figured in detail, and borne out by corresponding evidences on the four volcanoes most closely studied (La Malinche, Iztaccíhuatl, Popocatépetl, Citlaltépetl) Similar, but less complete, observations were made on a number of lesser peaks, which, although unglaciated today, show the signs of at least some of the late Pleistocene and Holocene events described.

A brave attempt is made to derive a palaeoclimatic explanation of the ground phenomena, which is, perhaps, less convincing than the results of the painstaking fieldwork. This is largely due to the very divergent views expressed in the climatological literature, so that any author is obliged to espouse one or other mutually exclusive theories.

An enormous Bibliography is provided of relevant literature, making this a valuable work of reference and a worthy successor to previous volumes in the series.

I. W. Cornwall

Hermetic dialogue

The Ash Wednesday Supper: La Cena de le Ceneri. By Girdano Bruno. Pp. 174. (Mouton: The Hague and Paris, 1975) 28 Dutch guilders.

The Ash Wednesday Supper (La Cena de le Ceneri) is one of six Italian dialogues written by Giordano Bruno for the Elizabethan court when he visited London in 1584–85. The underlying purpose of the dialogue was at once religious and political. Bruno hoped to achieve a union between England and France through a hermetic interpretation of the Christian religion. Historians of sixteenth century intellectual history have found Bruno's work interesting not only for this reason, but because it involves an extended metaphor grounded in Copernican astronomy. Professor Jaki's translation of *La Cena* is based on an edition by Giovanni Aquilecchia of 1955, rather than on the later and fuller edition by the same editor. Professor Jaki makes a very stern critic of Bruno's science in many of its details, and might have been a more sympathetic commentator had he not insisted on treating Bruno as something he was not, namely, a scientist in the modern sense of the word. It will cause no-one surprise to find that Bruno does not conform to the canons of modern scientific thought, and history is not likely to be much illuminated by showing how Bruno failed to do so. Does it matter that Bruno did not anticipate Olbers' paradox? Professor Jaki's translation will certainly make the work of Bruno more accessible to an English-speaking audience. The commentary propagates one or two old myths, such as that of the 50 astronomers engaged by Alfonso of Castile to revise the Ptolemaic astronomical tables. For the most part, the translation reads well, but there are a number of points where the very sense of the English should have drawn attention to a mistranslation. Lewis and Short will soon put right the translation offered for *Neque id sine lepore et gratia,* which can hardly mean "Not without a rabbit and thanks"! **J. D. North**

Porphyrins

Porphyrins and Metalloporphyrins. Edited by K M. Smith. New Edition based on the original volume by J. E. Falk Pp. xxiii+910. Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl. 275; $114.50.

THIS book will be warmly welcomed by all who work in the area of porphyrins and metalloporphyrins, as it brings up to date material covered by Falk, while changing the emphasis somewhat to cover synthetic aspects and applications of spectroscopic methods. Throughout, the authors have taken care to emphasise biological relevance, and a section dealing with porphyrin biosynthesis and catabolism is included. Apart from a lack of coverage of catalytic properties of metalloporphyrins, the choice of material and its distribution among the various chapters is excellent. The editor is to be further congratulated on the coordination and cross-referencing between various chapters and the absence of significant overlap from different contributors. The presentation is good with many helpful diagrams and formulae, but there is no distinction in the type-setting between different classes of subheadings and, in parts, this is confusing. The text contains an abundance of references to reviews and original papers up to late 1974.

The first section deals with nomenclature and general features of structure, stability and spectra, followed by a summary of recent developments in chemical synthesis. Section 2 contains chapters on biosynthesis (including an excellent account of the 'type III isomer problem') and degradation to bilins. A comprehensive account of the coordination chemistry of porphyrins including static and dynamic aspects is followed by a section on methods for determination of molecular structure. This contains a wealth of information on X-ray, mass spectrometric, infrared and Raman studies and, notably, a detailed discussion of nuclear magnetic resonance to porphyrins, metalloporphyrins and related compounds. Applications of electron spin resonance and Mossbauer spectroscopy are considered in subsequent chapters. Later sections deal with chemical reactivity (including developments in photochemistry) and with structural analogues of porphyrins. The book concludes with a detailed and useful section on laboratory methods.

At $114.50, very few workers in the field will acquire a personal copy and even libraries may prove reluctant purchasers — an unfortunate situation as such a useful book should be within reach of all interested in this field. The problem is only slightly mollified by the news that the Laboratory Methods section is to be published separately. It is to be hoped that a less expensive edition, perhaps in paperback, will soon be forthcoming. **S. B. Brown**

Understanding information processing

Understanding Language: An Information-Processing Analysis of Speech Perception, Reading and Psycholinguistics. Edited by Dominic W. Massaro. Pp. xii+439. (Academic: New York and London, September 1975.) $16.50; £7.90.

THIS volume comprises eleven chapters; Massaro is an author of six and most of the remainder are by students of his. The book has been edited purposefully so that the coverage and level of the chapters cohere. There are some highly positive general attributes of the book. A wide literature is competently summarised and the references are quite comprehensive as well as up-to-date. To focus a set of reviews of aspects of information processing on the central theme of language is worthwhile.

Massaro's introductory chapter is one of the weaker ones, expounding an overview from a basic flow diagram of the sort which shows how to get from sound waves to abstract memory using only three boxes. Such diagrams are at best a harmless device for distinguishing groups of experiments. Too often they confuse the hierarchical structure of knowledge with the psychological processes by which it is arrived at. This chapter seems to me superficial without being usefully introductory.

The speech perception section begins with a much needed chapter on basic articulatory and acoustic dimensions which facilitates access to a literature which usually takes these as read. Two chapters on acoustic features and temporal factors lead to a review of major theories. Three chapters on reading cover elementary feature processing, word recognition and eye movements. Too much space is devoted to the scholastic enterprise of compiling letter feature lists but the discussion of redundancy utilisation is thorough. Massaro denies the use of lexical redundancy, probably incorrectly. The closing section on psycholinguistics reviews matters adequately treated elsewhere but adds completeness to the volume.

This book offers few significant theoretical insights but it provides a very competent review of an important and mobile area of research and will be highly useful to the advanced undergraduate or specialist for two or three years.

Leslie Henderson

Pure gold

Lysosomes in Biology and Pathology, Volume 4. (North-Holland Research Monographs· Frontiers of Biology, Volume 43.) Edited by J. T. Dingle and R. T. Dean. Pp. xviii+614. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) $74 95; Dfl.180.00.

THE 'gold rush' following the discovery of lysosomes has now subsided. The easily accessible nuggets have been removed and it is now necessary to dig more deeply and systematically to reap rewards That they are still there is illustrated by several chapters in this volume, which report thorough analyses of old problems, such as the role of lysosomes in degradation of plasma proteins (G. Gregoriadis) and in intracellular protein turnover (H. L. Segal). Several chapters are devoted to lysosomes in different cell types (skeletal and cardiac muscle cells, blood platelets, arterial walls, skin, *Tetrahymena*) but these accounts are more than descriptive, embracing the participation of lysosomes in the physiology and pathology of the cells concerned. For example, blood platelets are rich in lysosomes, and the release of their enzymes contributes to the pathogenesis of inflammation; and storage of lipids in lysosomes of cells in arterial walls may be important in atherosclerosis.

Evidence is presented of heterogeneity of lysosomes within single cell types (M. Davies) and of multiple forms of lysosomal enzymes (R. T. Dean) Speculative chapters include those of C. M. Szego on lysosomes in nucleocytoplasmic communication and L. J. Ignarro on the regulation of lysosomal enzyme secretion. In these days when relevance is so much emphasised it is pleasing to see a beautifully illustrated account by Yolande Heslop-Harrison of enzyme release in carnivorous plants. As in previous volumes, the production is of high quality, with many illustrations, including well-reproduced half tones, and comprehensive author and subject indices. The quality of production is unfortunately reflected in the price. **A. C. Allison**

Starch structure

Starch and its Components. By W. Banks and C. T Greenwood. Pp. xi+342. (Edinburgh University: Edinburgh, 1975.) £10.00.

STARCH, particularly as it occurs in cereal grains, is the major carbohydrate food ingredient in the human diet. Yet the structure of the starch granule is still unknown and no one has synthesised a granule *in vitro*. Nevertheless a great amount is known about starch granules and particularly about the structure, behaviour and synthesis of its major molecular components, amylose and amylopectin

This book reviews the chemical nature of starch and its components, and their behaviour with enzymes The book is a highly biased presentation and as the authors state in the Preface "We make no apology for the fact that emphasis in this monograph is on our own work . " Not everyone will agree with all the authors' statements in the book, but I find the total coverage good and the many arguments and criticisms sound. A reader will carry away a fine sense of starch behaviour and a good feel for the structure and reactions of amylose and amylopectin Emphasis is placed on hydrodynamic behaviour and on the action of enzymes on amylose and amylopectin.

After a short introduction the book is separated into five major sections. The first deals with the fractionation of starch and the fine structure of its components. Several fractionation procedures are discussed and the chemical but mainly enzyme reactions of components are given. Emphasis is placed on the use of enzymes to elucidate starch molecular structure. A section of 43 pages is devoted to the reaction of starch and its components with iodine A section concerned with the conformation of amylose in solution provides a wide coverage of methods for conformational determination and possible interpretations It is agreed that the basic helical nature of amylose predominates in its complexes and is lowest, if existant at all, in neutral aqueous solution. Starch-degrading enzymes are treated by examining the action pattern of exo-, endo- and branching or debranching enzymes. Pitfalls can be numerous in the use of enzymes to deduce starch structure and many of these are clearly indicated. The final major section carries a discussion of the structure and biosynthesis of the starch granule. Attention is directed toward crystalline nature and orientation.

This is not a practical book. It does not discuss the characteristics of starch pastes nor of the preparation or use of starch or its modifications. The book is devoted to a discussion of the basic structure of starch and its components as interpreted by the authors. As such it is recommended to all those having an interest in the fundamental characteristics of starch. Biochemists will find the review useful and interesting **Roy L. Whistler**

Amateur astronomers' Bible

Astronomy: A Handbook. Edited by G. D. Roth. Translated and Revised by Arthur Beer. Pp. xviii+567. (Springer: Berlin and New York, 1975.) DM44.90, $18.50.

THIS is a revised and translated version of what was for long the German amateur astronomers' bible, *Handbuch für Sternfreunde*, described rather misleadingly on the dust jacket as "designed to fill the needs of both professional and amateur astronomers". It in fact sets out to encourage the amateur to make and reduce observations of professional quality and rigour, so that the two might be complementary.

The ground covered is naturally similar to that in other amateurs' handbooks such as J B. Sidgwick's *Observational Astronomy for Amateurs* and *Amateur Astronomer's Handbook*, but its approach is more sophisticated. The chapter on 'Applied Mathematics for Amateur Astronomers' requires maths of at least A-level standard, and several expressions elsewhere in the book would be clarified by the use of worked examples. Yet the handbook is curiously out of date in parts, the radio astronomy chapter contains circuit diagrams which abound in valves, and some of the definitions in this and other chapters have a ring of history about them. There are some strange omissions too, in what is overall a very comprehensive text. Narrow band and even white light observations of solar flares are not discussed; meteor triangulation is dismissed in one sentence, and meteor spectroscopy, where the amateur can contribute important results, is not even mentioned.

There are, however, some particularly good chapters which are not covered well elsewhere. 'Fundamentals of Spherical Astronomy' is an excellent compact reference to position and time measurements. The chapters on solar eclipses, lunar eclipses and artificial Earth satellites are also most informative, and that on 'Observation of the Planets' stresses the amateur's role in continuously monitoring changes in planetary detail. There is a useful and complete appendix; and although several of the original references in the text are necessarily in German, the very thorough bibliography suggests much in the way of alternative reading.

Although this book is unlikely to attain the popular appeal of Sidgwick's handbooks, it is a good reference work for the advanced amateur astronomer and will be welcomed by many.

Heather A. Couper

obituary

Rudolph Minkowski, an outstanding contributor to many areas of astrophysics, died on January 4, 1976, at the age of 80. As a staff astronomer at the Hale Observatories, and later at the Berkeley Radio Astronomy Laboratory, he pioneered the study of planetary nebulae, supernovae, and radio-emitting galaxies.

Minkowski was born in Strassburg and educated at Breslau. Although strongly interested in astronomy, he found the physics program more attractive at Breslau, and he took his Ph D in optics in 1921. After a year at Göttingen, he joined the physics group at Hamburg, where he rose to the rank of professor In 1935 the increasing Nazi repression drove Minkowski to leave his homeland. Encouraged by former colleague Walter Baade, who had preceded him to Pasadena, he moved to a research assistantship at the Mount Wilson Observatory, where his abilities soon earned him a regular staff position. In his 25 years with the telescopes of Mount Wilson, and later of Palomar Observatory. Minkowski became one of the World's leading investigators of the violent phenomena of the universe.

Supernovae remained one of Minkowski's central interests during his entire career He distinguished early between the two principal types of supernovae and studied the spectra of many individual supernovae in other galaxies In collaboration with Baade he studied the remnants of the few known supernovae that have appeared in our own Galaxy. Of particular importance was their thorough analysis of the Crab Nebula, an object whose importance in astrophysics has increased as it was discovered successively to be a radio source, an X-ray source, and a pulsar

After the discovery of discrete radio sources, Minkowski was a leader in their identification and interpretation. Again working with Baade, he identified some of the strongest radio sources as supernova remnants. Others were identified as disturbed galaxies; the original Baade–Minkowski interpretation of them as pairs in collision did not hold up, but their nature has continued to puzzle astrophysicists for two decades.

Another of Minkowski's long-term interests was the nature of planetary nebulae. In addition to his analysis of these objects, he set up a survey, using a 10″ telescope, that more than doubled the number of planetary nebulae known

Minkowski's greatest public service in astronomy, however, was his supervision of the National Geographic Society–Palomar Observatory Sky Survey. This incomparably valuable set of photographs has been made widely available in reproduction and is an essential part of the facilities of every astronomical library Its uniformly high quality is a result of Minkowski's painstaking care.

The study of supernovae and disturbed galaxies naturally led Minkowski to study normal galaxies as well. His pioneering study of internal motions in elliptical galaxies was superseded only a dozen years later, when a new generation of observing equipment became available. It was in his last observing run at the Palomar 200″ telescope that he determined the optical redshift of the radio source 3C 295, which remained the farthest point on the velocity–distance diagram of cosmology for 15 years.

After retirement from Mount Wilson and Palomar Observatories in 1960. Minkowski spent a year at the University of Wisconsin and then moved to Berkeley, where he retired again in 1965 Neither retirement had any visible effect on his output of scientific articles, which continued into the 1970s. Several generations of astronomers will remember the personal warmth that underlay his wisdom.

Ivan R. King

announcements

Awards

The Chemical Society, Washington, has awarded the 1975 Hillebrand prize to **Dr Ming-Chang Lin** for his work on and with chemical lasers.

Dr Harold A. Rosen has been awarded the first L M. Ericsson International Prize for his work on geostationary communications satellites.

The Institute of Physics has made the following awards for 1976·

Charles Vernon Boys Prize to **Professor S. D. Smith** of Heriot-Watt University, Edinburgh, for his contributions to the design of scientific instruments in solid state physics and in physical meteorology

Duddell Medal and Prize to **Mr G. N. Hounsfield** of EMI Limited, for his development in the use of X rays for the examination of three dimensional structures.

Glazebrook Medal and Prize to **Sir Montague Finniston** of the British Steel Corporation, for his leadership in the application of science to the large scale manufacture of steel

Guthrie Medal and Prize to **Professor A. Salam** of the Imperial College, London, and the International Centre for Theoretical Physics, Trieste, for his contributions to the theory of fundamental particles

Maxwell Medal and Prize to **Dr S. W. Hawking** of the University of Cambridge, for his contributions to theoretical astrophysics.

Rutherford Medal and Prize jointly to **Professor R. J. Blin-Stoyle** of the University of Sussex, and **Dr Joan M. Freeman** of the UKAEA, Harwell, for their work on β-radioactivity of complex nuclei

Appointments

Sir Kenneth Berrill, Professor C. C. Booth, Sir Alan Cottrell and **Mrs J. E. Floud** have been appointed to the Advisory Board for the Research Councils (ABRC).

Dr J. H. Humphrey, Deputy director of the National Institute of Medical Research, has been appointed Professor of Immunology at the Royal Postgraduate Medical School.

International meetings

April 1–2, **The Changing Environmental Conditions in Great Britain and Ireland** during the Devensian Cold Stage, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

April 20–23, **Human Reflexes and Motor Disorders,** Brussels (I.C.B., Chaussée de Waterloo, 715, B-1180 Brussels, Belgium).

May 14–15, **Platelets and Thrombosis,** Milan (Dr D. C. B. Mills, Specialised Centre on Thrombosis Research, Temple University, Health Sciences Centre, 3400 N. Broad St., Philadelphia, Pennsylvania 19140).

June 14–19, **4th International Conference on Marine Corrosion and Fouling,** Juan-les-Pins, France (Congress Secretariat, CREO-73, 77, rue de Sèvres, 92100 Boulogne-Sur-Seine, France).

July 13–17, **Biology and Taxonomy of the Solanaceae,** Birmingham (Mr David Langley, Conference Secretary, Department of Botany, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK).

July 26–28, **2nd European Colloquium on Hypothalamic Hormones,** Tübingen (deadline: April 15) (Professor Wolfgang Voelter, Chemisches Institut, Auf der Morgenstelle, 7400 Tübingen, FRG).

January 17–19, 1977, **The Geology, Mining and Extractive Processing of Uranium with Special Reference to Europe,** London (Deadline for abstracts: May 1) (The Secretary, Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

Person to Person

Scientist offers exchange of comfortable 3-bedroom house in Cambridge for a similar one in Paris from May 15 to July 30. (Contact Dr M. Levitt, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK).

Six months' notice is given of the possible use of plenary powers by the International Commission on Zoological Nomenclature in connection with the following names (see *Bull. zool. Nom.*, **32** (1976)):

260 *Dermacentor andersoni* Stiles, 1908 (Acarina: IXODIDAE): proposed conservation.

1772 *Ophiura* Lamark, 1801 and *Ophioderma* Müller and Troschel, 1840: revised proposals for stabilisation.

2097 *Ophiolepis* Müller and Troschel, 1840: proposed designation of type-species.

2112 CIRCINAE in Aves and Mollusca: proposals to end the homonymy.

2113 *Pterois zebra* Quoy and Gaimard, 1825 (Pisces: SCORPAENIDAE): proposed suppression.

2116 *Halecium* Oken, 1815 (Coelenterata: Hydroida): Proposed validation: *Thoa* Lamouroux, 1816, proposed suppression.

2119 *Lecanium acuminatum* Signoret, 1873 (Hemiptera: COCCIDAE) proposed designation of neotype.

2120 *Cystioceras* Börner, 1912 (Insecta: Collembola): proposed suppression.

Comments within 6 months to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London SW7 5BD, UK.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office The section will include exchanges of accommodation, personal announcements and scientific queries We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

United States Department of the Interior: Geological Survey. Water-Supply Paper 2030· Summary of Floods in the United States During 1969. By J. K. Reid. Pp viii + 173. Water-Supply Paper 2153. Quality of Surface Waters of the United States, 1970. Part 3. Ohio River Basin. Pp. x + 444 (Washington, D-C· S Government Printing Office, 1975.) [1212

Royal Ontario Museum. A Selected Bibliography on Mercury in the Environment, with Subject Listing. By Susan Robinson and W. B. Scott. Pp. 54. (Life Sciences Miscellaneous Publication.) $1.25. Life Sciences Contributions. No 99 A New Genus of Freshwater Triclad from Tasmania, with Reviews of the Related Genera *Cura* and *Neppia* (Turbellaria, Tricladida). By Ian R. Ball. Pp. 48. $2. No 100 A Revision of the Latipinnate Ichthyosaurs of the Lower Jurassic of England (Reptilia· Ichthyosauria) By C. McGowan. Pp. 30. $1.50 No. 101· Redescription of Type Specimens of the Bryozoan *Heterotrypa* from Upper Ordovician Rocks of the Credit River Valley, Ontario, Canada. By Madeleine A. Fritz. Pp. 30. $1.75. No. 102. Fauna and Correlation of the Ravenscrag Formation (Paleocene) of Southwestern Saskatchewan. By L. S. Russell. Pp. 53. $2. No. 103: Tertiary Mammals of Saskatchewan Part 3: The Miocene Fauna. By John E. Storer. Pp 124 -$4. No. 104. Observations on the Biology of Some Fhodesian Bata, Including a Key to the Chiroptera of Rhodesia By M. B. Fenton. Pp. 27. $1 25. No. 105 The Morphology, Karyology and Taxonomy of a New Freshwater Planarian of the Genus *Phagocata* from California (Platyhelminthes· Turbellaria) By Ian R. Ball and N Gourbault. Pp. 19 $1.50 Occasional Papers. No 26: Thermal Relations of the Neotropical Frog *Hyla labialis* (Anura· Hylidae). By D. Valdivieso and J. R. Tamsitt. Pp. 12. No 27. Chromosomes of Fifteen Species of Bats (Chiroptera) from Kenya and Rhodesia. By R. K. Paterson and David W. Nagorsen. Pp. 16. No. 28· Karyotypes of Six Species of Bats (Chiroptera) from the Dominican Republic. By David W. Nagorsen and R. L. Paterson. Pp 8 (Toronto. Royal Ontario Museum, 1974 and 1975.) [1512

Peabody Museum of Natural History, Yale University, Bulletin 40: On the Biology of Cosmine. By Keith Stewart Thomson. Pp. 59 (New Haven, Conn· Peabody Museum of Natural History, 1974.) [1512

Norsk Polarinstitutt. Skrifter Nr 162· The Ordovician Trilobites of Spitsbergen. II. Asaphidae, Nileidae, Raphiophoridae and Telephinidae of the Valhallfonna Formation By R. A. Fortey. Pp. 207 (41 plates). (Oslo. Norsk Polarinstitutt, 1975.) [1512

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils Technical Paper No. 25: Cultivation and Fertilizer Effects on the Growth and Foliage Nutrient Concentrations of *P. radiata*, *P. pinaster* and *P. caribaea* on Three Soil Types at Anglesea (Victoria) By M. Raupach, A R. P. Clarke, B. F. Gibson and K. M. Cellier. Pp. 20 (Glen Osmond, SA· CSIRO, Division of Soils, 1975.) [1512

Environment Canada: Fisheries and Marine Service. Technical Reports. No. 581· Identification, Host Sites and Biology of Parasites Infecting Juvenile Atlantic Salmon (*Salmo salar*) in the Miramichi River System. By G M Hare and M. D. B. Burt. Pp. 34 No. 582. YRAREA—a Program to Demonstrate Effects of Exploitation on a Contagiously Distributed Shellfish Population. By L. E Gales and J. F Caddy. Pp. 79. (St. Andrews, NB· Research and Development Directorate, Biological Station, 1975) [1512

Geological Survey of Canada The Geology of Long Beach Segment, Pacific Rim National Park and Its Approaches By A H. Lang and J. E. Muller. Pp. 56. $2.40. Bulletin 249: Upper Cretaceous Stratigraphy, Yukon Coastal Plain and Northwestern Mackenzie Delta. By F. G. Young. Pp. xii + 83. $7.20. Paper 74–29· Bylot Island Map-Area, District of Franklin. By G D. Jackson nad A. Davidson. iii + 12. $2.40. Paper 74–48· Major Rock Units of the Morin Complex, Southwestern Quebec. By R F. Emslie. Pp. iv + 37. $4.20. Paper 95–1, Part C· Report of Activities, Part C. Pp. vi+371. $6. Paper 75–5. Current Research in the Geological Sciences in Canada, 1974/75. Compiled by Thomas E. Bolton. Pp. 107. $2.40. Paper 75–32: Hesquiat Formation (New)—Neritic Channel and Interchannel Deposit of Oligocene Age, Western Vancouver Island, British Columbia. By J. A. Jeletzky. Pp. 95 (6 plates). $4 20. (Ottawa: Information Canada, 1975.) [1512

United States Department of the Interior: Geological Survey. Professional Paper 437–D: Land Subsidence Due to Ground-Water Withdrawal, Arvin-Maricopa Area, California. By Ben E. Logfren. Pp. iv+55. Professional Paper 827. Geology of the Sierra Foothills Melange and Adjacent Areas, Amador County, California. By Wendell A. Duffield and Robert V. Sharp. Pp. iii + 30 + 1 plate. Professional Paper 857: Diagenesis and Stratigraphy of the Lisburne Group Limestones of the Sadlerochit Mountains and Adjacent Areas, Northeastern Alaska. By George V. Wood and Augustus K. Armstrong Pp.vi + 47 + 12 plates. $2.25. (Washington, DC. US Government Printing Office, 1975.) [1512

The Fishery Limits Off Iceland—200 Nautical Miles. Pp. 48. (Reykjavik Ministry of Foreign Affairs, 1975.) [1612

National Hydatids Council. 15th Annual Report and Statement of Accounts for the year ended 31 March 1975. Pp. 24 (Wellington, New Zealand: National Hydatids Council, Ministry of Agriculture and Fisheries, 1975.) [1612

United States Department of the Interior: Geological Survey Bulletin 1395–A Changes in Stratigraphic Nomenclature by the U S. Geological Survey, 1973. By George V. Cohee and Wilna R. Wright. Pp. iii + 68. 95 cents. Water-Supply Paper 2122: Surface Water Supply of the United States, 1966–70. Part 8. Western Gulf of Mexico Basins. Vol. 1. Basins from Mermentau River to Colorado River. Pp. x+1144. Professional Paper 433–P: Discharge and Flow Distribution, Columbia River Estuary. By G. A. Lutz, D. W. Hubbell and H. H. Stevens, Jr Pp. iv + 31. (Washington, DC: US Government Printing Office, 1975.) [1612

Australia· Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Animal Health, 1974. Pp. 125. (Melbourne. CSIRO, 1975.) [1812

Australian Venomous and Poisonous Fishes. By R. V. Southcott. Pp 20. (Mitcham, South Australis: R. V. Southcott, 2 Taylors Road, 1975.) US$2.00. [1812

United States Department of the Interior: Geological Survey. Bulletin 1397–A· Mineral Resources of the Teton Corridor, Teton County, Wyoming. By J. D. Love, J. C. Antweiler and F. E. Williams. Pp. vi + 51. (Washington, DC: US Government Printing Office, 1975.) [1912

Institut National de la Recherche Agronomique. Service d'Étude des Sols et de la Carte Pédologique de France. Carte Pedologique de France a 1/100,000—Moulins. Notice Explicative Par J. C. Favrot. Pp. 175. (Versailles, Centre National de Recherche Agronomiques, 1974.) [2212

An Intercomparison of Some Current Meters, III. (Report on an experiment carried out from the Research Vessel *Atlantis*, II, August-September 1972, by Workshop Group on Continuous Current Velocity Measurements. Final Report of Working Group 21.) (Unesco Technical Papers in Marine Science, No. 23) Pp. 41. (Paris. Unesco, 1975) [2212

Canadian Hydrographic Service & Geological Survey of Canada The Gravity and Magnetic Fields of Atlantic Offshore Canada. By Richard T. Haworth and J. Brian MacIntyre. Pp. iv + 22 (Marine Sciences Paper No. 16; Geological Survey of Canada Paper 75–9.) (Ottawa· Information Canada, 1975.) $3.60. [2212

United States Department of the Interior: Geological Survey. Bulletin 1409. A Summary of Oil and Gas Production and Reserve Histories of the Appalachian Basin, 1859–1972. By Betty M. Miller. Pp. iv + 36. Water-Supply Paper 1827–G. Effects of Organic Solutes on Chemical Reactions of Aluminium. By C. J Lind and J. D. Hem Pp. v + 83. Professional Paper 769· Bedrock Geology and Ore Deposits of the Palmer Quadrangle, Marquette County, Michigan. By Jacob E. Gair. Pp. v + 159 + 8 plates. Professional Paper 792: Reconnaissance Geology of Chichagof, Baranof, and Kruzof Islands, Southeastern Alaska. By Robert A. Loney, David A Brew, L. J Patrick Muffler, and John S. Pomeroy Pp. v + 105 + 4 plates Professional Paper 917: A Numerical Model of Material Transport In Salt-Wedge Estuaries. By H. B. Fischer, *et al* Pp. v+36 (Washington, DC. Government Printing Office, 1975.) [2212

The International Exchange and Testing of Cassava Germ Plasm (Proceedings of an Interdisciplinary Workshop held at CIAT, Palmira. Colombia, 4–6 February 1975.) Edited by Barry Nestel and Reginald MacIntyre. Pp. 74. Integrated Approach to Local Development. (Report of an Interdisciplinary Seminar, Makari, Philippines, 31 March—3 April 1975.) Edited by Marilyn Campbell. Pp. 52 (Ottawa L International Development Research Centre, Box 8500, 1975.) [2312

Smithsonian Contributions to Zoology, No. 207: Anatomy of the External Nasal Passages and Facial Complex in the Delphinidae (Mammalia Cetacea). By James G Mead. Pp. iv + 72. (Washington. DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [2312

Fisheries Research Board of Canada. Annual Report for 1974. Pp. 18. (Ottawa: Environment Canada, Fisheries and Marine Service, 1975.) [2312

United States Department of the Interior· Geological Survey. Water-Supply Paper 1608–0. Reclamation by Tubewell Drainage in Rechna Doab and Adjacent Areas, Punjab Region, Pakistan By Glenn T. Malmberg. Pp. iv + 72 + 5 plates. Water-Supply Paper 2104: Surface Water Supply of the United States, 1966–70 Part 2: South Atlantic Slope and Eastern Gulf of Mexico Basins. Vol. 1: Basins from James River to Savannah River. Pp. ix+1018. $6.60. (Washington, DC: US Government Printing Office, 1974 and 1975.) [2312

nature

April 1, 1976

Too much British common sense

ASK a hardened British government watcher how he feels about the documents pushed at him, and his response will generally be equivocal, even though what he reads is often a model of clarity, coherence and common sense. The discussion paper *Future World Trends,* released last week by the Cabinet Office (HMSO, 60p), has, however, a big enough element of common sense to evoke profound worry, rather than mere ambivalence. For common sense is conservative; and the implicit no-change theme running through the content gives it an ostrich-like perspective.

The document is the product of an interdepartmental committee of (regrettably anonymous) government scientists chaired by Dr Robert Press of the Cabinet Office, and the committee's objective (apart from stimulating "informed discussion") was to make its own assessment of projections on population, food, energy, mineral resources and pollution and to "help establish the position to be taken by the United Kingdom in international matters".

Future World Trends is worrying for far more important reasons than the obvious one that the subject matter is gloomy. We knew that already. But, even allowing for its limited size and scope (and the immensely irritating lack of references), the product is below standard. Not only is it more interesting for what it leaves out, or refuses to emphasise, than for what it includes; the tone it adopts as a consequence also makes it far from suitable as a basis for genuine "informed discussion".

Amid all the interesting (if obvious) points made about exponential population growth, for example, the only real tilt made towards the now widely accepted view that high population growth is the consequence and not the cause of poverty is a mere mention of the rather woolly view endorsed at the 1974 World Population Conference in Bucharest that "voluntary reductions are primarily dependent on changing social attitudes which are more likely to come about as a result of improved living conditions". Nothing is ventured regarding the structural changes which might be necessary to allow just such an improvement.

On theoretical food needs early next century, the document says these could be met but are not in fact likely to be. But the crucial explanation is bland: there are "economic, social and political barriers to the optimal policy of concentrating the expansion of output"; "similar obstacles" restrict "equitable global distribution"; growth in the consumption of (inefficiently produced) animal protein foods in rich communities will continue. As with population, the implicit conclusion is not drawn out, namely, that there is something wrong with the global economy and its capacity to allocate resources effectively. The need for a "massive transfer" of resources to what it calls (wrongly) the "developing countries" is mentioned—but is then largely discounted.

The committee's discussion of mineral resources highlights very well the orientation which makes for its difficulties on such matters. The price mechanism, it explains, operates to bring supply and demand for mineral resources into equilibrium. Because of it, there are actually "no hard and fast physical limits to resources"; the limits are "economic and technological". But the document (thoughts of OPEC?) adds, without elaboration: "national questions of environment and security of supplies and international constraints will undoubtedly affect the availability of raw materials". Later, on the broader use of nuclear energy, nothing is said of the proliferating international trade in nuclear technology which is its precondition. The prospect of nuclear blackmail, or indeed anything else which might upset present geopolitical arrangements and global economic patterns, is apparently not relevant.

What is relevant, it seems, is the 200th anniversary of Adam Smith. A look at the all-too-brief section headed "Economic aspects" highlights once again the ubiquitous price mechanism. This, we are told, will in general maximise welfare "subject to the distribution of income and wealth being socially and politically acceptable"; government intervention may be necessary in the event of "market failure". Yet it is precisely the unacceptability of the existing distribution which identifies the weakness of the market mechanism; and it is "market failure" (did it ever succeed?) which has spawned documents like this and its predecessors.

There is no reference to the idea that, without uninhibited expansion (which is what the debate is all about), and probably even with it, the market mechanism alone cannot produce anything other than an uneven allocation of resources; nor of the idea that there must be some sort of redistributing agency if the underdevelopment which is the corollary of the industrialised countries' development is to be forestalled. Yet the so-called "developing countries" (to the extent they are discernible as that—is Iran any longer comparable to Tanzania?), with few exceptions, are not developing at all; and the population and food problems will remain until real development takes place.

It seems incredible that a document looking at the next 30 years should evade all these points about the international economy and international politics. Scientists know that nowadays they will be judged for their public comments (or lack of them) regarding society as much as for their comments within their specialities—especially when they are participating in the gloom game. Yet this committee has largely failed to acknowledge that the real problems of the future will most likely be essentially political ones.

The necessary transfer of resources to ameliorate the population and food problems will demand sacrifices; so will the commitment of resources to solving these and other problems (energy, pollution). Such an awkward reassessment of priorities demands not only leadership but also authority—or, put another way, obedience. The sacrifice may therefore be, for the few countries which have elements of it, democracy itself. And it is a measure of the problem that it is democracy which offers the best chance that the consequence of an agonising choice might lead to success. The committee has chosen not to question the survival of the west's "business civilisation". Politics, after all, wasn't within its remit. So its ostrich-like perspective has contrived a camel. And a rather ordinary one at that.

An immunological approach

Barry Bloom, professor of microbiology at New York's Albert Einstein College of Medicine, looks at the possibilities for preventing parasitic diseases in cattle

WITH projections that world population will increase from 4,000 million to about 7,500 million by the end of the century, and an estimated 500 million people currently suffering from malnutrition, the magnitude of the world food crisis has finally touched the consciousness of people everywhere. Attention has been focused primarily on the green revolution and the need for increasing production of food grains; consequently the need for increasing production of meat and cattle has for the most part been overlooked.

The present situation is grim. While reliable statistics are virtually nonexistent, millions of head of cattle die of parasitic diseases each year, and more than 700 million are estimated to be at risk. In Africa alone, at least 7 million square kilometres of grazeable land, capable of supporting 120 million head of cattle, remain largely unproductive, chiefly because of tryanosomiasis and East Coast fever. The use of pesticides, while clearly important, has been inadequate to stave off these diseases, which are carried by the tsetse fly and ticks respectively. Effective drugs either do not exist or are too expensive for general use.

As a consequence of the enormous losses in potential food resources, there has recently been some discussion of the possibility of developing vaccines for immunizing cattle against diseases caused by blood parasites. In particular, the Rockefeller Foundation has sponsored a series of meetings to discuss the prospects.

Advocates of an immunological approach to control of parasitic diseases draw encouragement from two simple observations. First, if there were no immunity to such diseases, there would probably be no cattle in most of sub-Saharan Africa. Second, although mortality from these diseases is distressingly high, animals which survive infection are often resistant to subsequent reinfection. In fact, animals native to Africa, such as the n'dama, appear to be more resistant to parasitic diseases than species introduced from Europe. The general hope is that, with better understanding of the immune mechanisms required for protection, better characterisation of the parasite antigens required for resistance, and the use of immunological adjuvants—which are used remarkably infrequently in parasitic immunology—an immunological approach to control of these diseases could be realistic.

Although research has so far been limited, there have already been some promising results. Vaccines have been developed for protecting cattle against three parasitic diseases (babesiosis, anaplasmosis and East Coast fever) and immunisation has been found to offer protection against three others in laboratory animals (malaria, South American and African trypanosomiasis).

Even beyond our current wealth of ignorance of the specific aspects of host-parasite relationships, there exist serious problems which limit real progress in this area. A major problem is money. According to recent figures from the World Health Organisation, for example, in 25 African countries for which details are available, the average annual health budget is $1.17 per capita, and in 8 countries it is less than $1 per capita. In seven of those countries, the health budget is less than 5% of the total government budget. If those figures represent levels of support for control of human disease, the prospects for control of animal diseases by the developing countries themselves are clearly dismal. Moreover, the more developed countries hardly seem to be concerned with these problems—in recent years, there has been a general reduction in bilateral and international assistance programs in health-related areas.

A further problem is the increasing criticism of meat production and consumption in the West. While this campaign has relevance to grain-intensive cattle production in the developed countries, there is a danger that it may inhibit development of important food resources in developing nations where grain-intensive feeding is neither necessary nor possible. In sub-Saharan Africa, for example, cattle feed on low grade brush and natural vegetation, on lands generally too poor to support cultivated crops.

Promising developments

In spite of those problems, however, there have been some promising developments against the following widespread parasitic diseases.

- The most enigmatic and biologically fascinating of the animal parasite diseases is East Coast fever, caused by *Theileria parvum*, a virtually unclassifiable tick-borne parasite. The disease, which takes the form of a galloping proliferation of lymphocytes, not unlike leukemia, is responsible for the death of half a million cattle a year. The extraordinary aspect of the disease in cattle is that the parasite grows only in lymphocytes, and appears to act in a manner similar to cancer-causing viruses in tranforming lymphocytes into continuously growing cell lines.

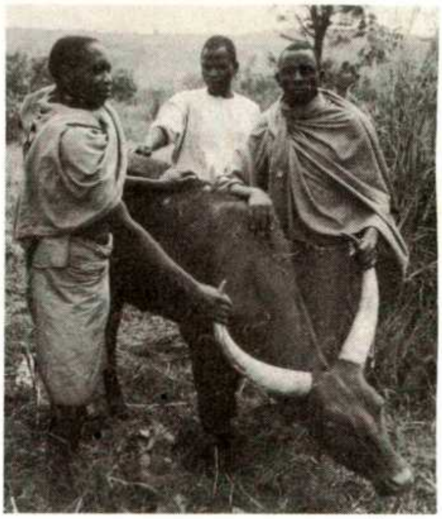


Photo: Popperfoto

Tsetse fly infection in Uganda

There has been progress in producing immunity in cattle in two ways: by infecting them with ticks, followed by antibiotic treatment, and more recently, by immunising them with infected lymphocyte cultures. The hope is that the parasite can be isolated from infected lymphocyte cultures, and the protective antigens purified and characterised.

- A million young cattle die each year from another tick-borne disease, anaplasmosis. It is caused by a family of rickettsiae which infect and multiply within mature erythrocytes. An attenuated vaccine has been produced by subjecting the parasite to gamma radiation and passing it repeatedly through an unnatural host, the sheep. Striking protection against tick infection has been observed in small scale experimental trials in the United States, and more extensive field trials are under way in Mexico and Columbia.
- A third tick-borne disease, babesiosis, is responsible for the death of about a quarter of a million cattle a year. The parasite is related to the malaria parasite, and similarly multiples in and destroys erythrocytes. An attenuated organism has been produced by passing the parasite through splenectomised cattle, and a highly effective vaccine, currently in use in Australia, has been developed.
- African trypanosomiasis, the most serious of the animal diseases, is responsible for the death of 3 million cattle a year. The animal disease and human sleeping sickness, both of which are caused by parasites carried by the tsetse fly, are clinically very similar. They represent a very difficult immunological problem because the parasite

has developed a highly effective method for eluding the immune system of the host, known as 'antigenic variation'. The disease is characterised by waves of illness, reflecting rapid growth of the parasite, followed by remission engendered by an immune response, followed by sequential emergence of antigenically different organisms.

Because the organism has not yet been grown in culture, it is not known by what genetic or regulatory mechanism it manages to change its surface characteristics to evade the immune system. Two different lines of research for potential vaccination are, however, being followed. The first would involve modifying the immune system of the host so that it would react quickly to wipe out the second appearance of the organism before it reaches sufficient numbers to produce disease. Following the recent isolation of the major antigenic surface molecule, the second would involve the production of a "cocktail" vaccine containing a mixture of antigens from the most common variants to combat the different forms of the organism. Both require better characterisation of the organism and its antigens.

● There are many similarities between the animal and human parasitic diseases, such as Chagas disease and malaria, and there are consequently common research problems and strategies. Chagas disease, caused by the organism *Trypanosoma cruzi,* affects 30 million people, chiefly in Latin America. Ten per cent of its victims, virtually all children, die from an acute disease, while the rest develop a chronic illness with invasion and destruction of heart and smooth muscle. It frequently ends in sudden death. Although there is no effective treatment at present, an irradiated vaccine has been produced from parasites grown in animal cell cultures. It provides virtually 100% protection to massive challenge in susceptible mice, which are killed by a single virulent trypanosome.

● Two hundred and fifty milion people now suffer from malaria worldwide, and at least a million people die from the disease in Africa alone. Two successful types of experimental vaccines against different forms of the organism have been produced, but practical limitations on the quantity of these vaccines preclude either of them from being useful on a large scale. One line of research is aimed at defining the nature of the protective antigens so that synthetic antigens could be developed as potential vaccine material.

There are major scientific questions to be understood and problems to be overcome that will require contributions by molecular biologists and geneticists as well as parasitologists and immunologists, and at this stage even if increased support for research were forthcoming, it would be foolish to guarantee that inexpensive and effective vaccines and drugs would result. Even if some were to be developed, there would remain two further serious problems. Although effective vaccines, particularly against African trypanosomiasis, would offer the possibility of opening vast areas in the tsetse belt to cattle production, without thoughtful assessment of the ecological consequences and intelligent regulation, marginal areas of the Sahel and tropical Africa could be destroyed by overgrazing. The second problem is the lack of an effective health infrastructure in the developing countries. There is a critical need for developing health services and training adequate health manpower to provide basic information and care for the health needs of the human and animal populations.

At the very least, it is worth considering what could be accomplished simply by supporting studies on the epidemiology of the animal parasitic diseases. It is an activity which could involve large numbers of untrained young people in an important scientific and educational enterprise. It would serve to develop a pool of talent from which the best could be selected for more advanced training in the health sciences and services. ☐

USA

Behind the politics of influenza

The idea of the United States government footing the bill to prevent the American population falling sick makes President Ford's request for funds to attempt a mass inoculation against flu something more than a medical issue in this election year. From Washington, Colin Norman gives the background

ON FEBRUARY 3, a young army recruit stationed at Fort Dix, New Jersey, came down with what seemed like a typical case of influenza. But the next day, while on a training march, he collapsed and died. His death sparked off an extraordinary series of events, culminating last week in the launching of the most extensive and controversial mass inoculation campaign ever undertaken in the United States.

The cause of the soldier's death turned out to be pneumonia, but a virus isolated from his respiratory tract immediately set alarm bells ringing in public health laboratories around the world. It was found to be a strain of influenza virus, commonly found in swine, which had not been involved in human infections for nearly 50 years. The virus, moreover, is very similar to the strain which caused the most serious flu pandemic in modern history, the 1918–19 outbreak which swept around the world killing some 20 million people.

Though only 12 other cases of swine flu were subsequently confirmed at Fort Dix, a massive monitoring effort on the base found antibodies to the flu strain in the blood of some 500 recruits, suggesting that they had been infected with swine flu during the outbreak. If the young recruit hadn't died, the outbreak would simply have been put down to the so-called Victoria/A strain which has been widespread in the United States this winter, but the isolation of the swine virus may have provided advance warning of a possible new flu pandemic next winter.

Because swine flu hasn't been abroad in the human population since the late 1930s, nobody under the age of 50 has any antibodies to guard against it. And it is likely that even those who do have antibodies are not protected against the disease. Thus, if the Fort Dix outbreak is not just a freak, isolated event, the virus could spread like wildfire through an unprotected population. Dr Edwin Kilbourne, a leading flu researcher at Mount Sinai School of Medicine in New York, said last week, for example, that "our thought is that probably the virus is seeded now in foci around the country, and when winter comes we will see it again".

For several weeks after the virus was isolated and identified, committees of scientists and government officials met at the US government's Center for Disease Control in Atlanta, Georgia, to decide what, if anything, should be done to counter the potential threat. Last week, they decided to recommend a massive inoculation effort against the virus, and President Ford appeared at a press conference, flanked by two of the most well known scientists in the United States—Jonas Salk and Albert Sabin—to announce that he is requesting $135 million from Congress for a crash programme to inoculate every man, woman and child in the United States by the end of November.

The decision was immediately controversial. Officials from the World Health Organisation expressed surprise, some scientists argued that since there's no definite evidence that the Fort Dix outbreak has spread there is no need for a massive inoculation campaign, and there have even been dark hints that the whole thing is an election-year ploy to attract votes. Nevertheless, it can reasonably be argued that Mr Ford had little choice in the matter.

One-jump-ahead virus

The influenza A virus is an amazing organism, which has managed to evade man's attempts to keep it in check for three decades. Its ability to cause epidemics lies in its extraordinary capacity to stay one jump ahead of the body's natural defence mechanisms by changing its genetic makeup every few years. (Two other classes of influenza virus, designated B and C, are rather less elusive, however.) When a person is infected by an influenza virus he develops antibodies which will give immunity from subsequent reinfection, but if the virus undergoes a slight genetic change, the antibodies often won't recognise it and the virus can slip past the body's defences and cause disease. The flu virus's ability to change its character is also the reason why attempts to develop vaccines against flu have been relatively disappointing —vaccines developed against one year's flu virus are often ineffective against a modified strain which may appear the following year.

Aside from those small elusive changes, the influenza virus is also capable of major genetic shifts. Every so often, a radically new variation of influenza virus emerges and, because there is no natural resistance to it at all, it can cause major pandemics. The last such pandemic occurred in 1968 from a strain first isolated in Hong Kong (hence its popular designation as Hong Kong Flu); another worldwide epidemic occurred in 1957 from a strain which first appeared in Asia.

President Ford : requesting $135 million

If, as seems to happen, pandemics appear in roughly 10-year intervals, a radically new flu strain can be anticipated in the late 1970s. The Fort Dix swine flu virus may be that strain. If so, it would be the first time that the world has had advance warning of a pandemic, and that warning could provide time for preventive action. But the evidence is far from conclusive; hence the controversy over Mr Ford's decision to launch a mass inoculation campaign.

Following the isolation of swine flu virus at Fort Dix, a massive study was conducted to see whether the virus is showing up anywhere else in the United States. No other cases were found, and the virus also seems to have disappeared at the army camp. It is therefore possible that the outbreak was a freak event which suddenly ended through unknown causes. But, on the other hand, it is possible that the virus has been seeded in the population and will re-emerge during next winter's flu season.

Kilbourne notes, for example, that "historically we have never recognised a new (flu virus) variant without a major pandemic following in its wake", and he added that the Asian and Hong Kong flu epidemics "smouldered before they took off" in the United States. Dr Robert B. Crouch, professor of infectious diseases at Baylor College of Medicine, is also not surprised that the Fort Dix outbreak seems to have disappeared. He pointed out that the Asian flu epidemic was first spotted in the United States as an isolated outbreak in Iowa which died off, followed by another isolated outbreak in Pennsylvania, which also disappeared; two months later, the full-fledged epidemic began to appear.

Time barrier

The problem now, however, is that there's not enough time to wait and see whether other outbreaks of swine flu occur before taking evasive action. Production of the 200 million flu shots which will be required to inoculate the entire US population must begin immediately or the programme won't be completed before next winter. Thus Ford asked Congress last week for funds to begin manufacturing vaccines from the Fort Dix strain as soon as possible, with the goal of inoculating high risk people (the elderly and infirm) by late summer, and the rest of the population by November.

Some scientists have argued, however, that even if the virus does reappear next winter, there is no need to mount a major campaign against it now. Dr Sidney Wolfe, head of Ralph Nader's Health Research Group, argues, for example, that the analogies which have been drawn between the Fort Dix strain and the swine flu virus which caused the 1918 pandemic have been greatly overstated. The huge death toll from the 1918 outbreak resulted from secondary infections such as pneumonia, which can now be cured by antibiotics, and so there's no reason to suppose that a new flu pandemic would be as devastating. It should be pointed out, however, that the Hong Kong flu pandemic killed some 20,000 people in the United States and cost more than $3,000 million in terms of lost work and medical expenses, antibiotics notwithstanding.

One particularly disturbing, but inevitable, factor is that the vaccine now being developed will only be effective against the Fort Dix swine flu strain, but the Victoria/A strain and an influenza B strain are also expected to reappear next year. People will therefore be receiving flu shots but can still expect to get a variety of influenza, a fact which won't increase public confidence in medical science.

A more alarming possibility, which cannot be dismissed out of hand, is that an entirely new flu strain could emerge in the southern hemisphere during the next few months as the harbinger of a different flu pandemic. In that case, people in the United States could be getting immunized against the wrong pandemic strain.

Be that as it may, President Ford was presented with a problem for which there is really only one solution. Though the evidence is sketchy, it is possible that a pandemic flu strain has been isolated in time to take precautionary action. Mr Ford could gamble that the virus won't reappear and do nothing, in which case he would bear a heavy responsibility if the virus did show up again, or he could ask Congress for $135 million as an insurance policy.

Though those involved in the decision vehemently deny that politics played any part, it cannot be denied that if Ford refused to initiate an inoculation program and swine flu became rampant in October, the effect on the November elections could be decisive. By the same token, it is unlikely that Congress will deny the request for the funds.

- A statement released last week by Britain's Department of Health in response to developments in the United States said that on present evidence its advisory group on influenza would not be recommending vaccination. The group suggests, however, that it would be a wise precaution to incorporate the new virus into anti-flu vaccine.

BRITAIN

ALMOST two years after it was officially adopted as a run-up to Britain's fast breeder reactor programme, the Steam Generating Heavy Water Reactor (SGHWR) made the headlines again last week with a report that a starting date has at last been set for the construction of the first commercial installation at Sizewell in Suffolk.

Even in passing that historic milestone, however, the two organisations with major development interests in the new reactor were still displaying the sort of confusion and uncertainty that has dogged the project's progress since it was first mooted at the beginning of the decade. While the Department of Energy readily confirmed that the first sod will be turned in late 1977, both the Nuclear Power Company (NPC), responsible for producing the reactors planned for Sizewell and Torness, and its main customer, the Central Electricity Generating Board (CEGB), denied that a firm date had been set.

If not cagey, NPC is at least determined not to tread on sensitive CEGB toes, seeming eager to stress only that virtually every figure ever mentioned in connection with the project has little more solid basis than the paper it is written on. NPC says that reports putting the price of the first two SGHWRs (totalling 4,000 MW) at up to £1,500 millions cannot be taken seriously as they have not outlined the separate elements contributing to the total sum or considered whether they are capital or recurring costs. With so little being said, it is an understandable omission.

The CEGB's sensitivity is, nonetheless, forgiveable in view of the fiasco that has accompanied the development of the ill-fated Advanced Gas Cooled Reactors (AGRs). Tight CEGB lips last week parted just wide enough to explain that a nominal sum spent at the drawing board could later save millions on site modifications—a lesson expensively learned with the Dungeness B AGR. So, whatever the Department of Energy may proclaim, there will be no firm starting date from CEGB or NPC until final design details have been submitted by NPC, accepted by the CEGB, and passed by the Nuclear Installations Inspectorate.

● The academic year 1974-5 will not be remembered as a great year for universities; but at least they all stayed open. That, roughly, is the conclusion of the University Grants Committee's (UGC) report for last year published recently (Cmnd 6435, 50p). Supplementation, in bits and pieces, of the basic grant could not soften entirely the cut in real terms of about 8% in the recurrent grant that UGC handed on to universities.

Many of the economies that were made in that year are not repeatable, or indeed maintainable for much longer. 500 academic posts were frozen. The consequences, the lucidly-written report points out, are "a staff distribution growing increasingly at variance with student distribution; lack of new appointments [for] promising young graduates, and failure to develop new areas of study which should be their province". Mobility is being blocked as tenured staff cling to their posts.

Building programmes, apart from those to bring medical student numbers up to 4,100 by 1979 and those to house computers, are almost at a standstill. Nothing was done for libraries, twelve of which already have proposals for expansion and another ten of which are expected to look for expansion soon. More radical proposals to alleviate library problems are likely soon.

One trend which took UGC by surprise was that a number of universities want to start new courses in food science and technology, and nutrition. The UGC wasn't convinced that there was that much need, but it might do a bit more enquiring, as those in food seem to say the opposite.

A disturbing aside to the report was that students are now arriving at universities well-motivated, with good paper qualifications but lacking some of the basic skills. Modern languages, science and technology seem to be the worst sufferers, and remedial courses are taking up valuable university time. The reasons are complex, no doubt; the lack of well-qualified school teachers, failure of universities to respond to curriculum changes in schools, too much froth and not enough staple diet in some school courses—these are all likely contributors. The Education and Science Minister himself appealed to school heads just last week to try and boost by a tenth the numbers of students with A level passes in science and maths going to universities.

● Discouraging news for the UK fishing industry. In spite of the British Trawler Federation's pressures, which have already led Foreign Secretary James Callaghan to protest in Brussels about the inadequacy of the 12-mile national fishing limit called for by the EEC Commission, it has become clear that the industry is not going to get the 100-mile limit it wants.

Facing the Select Committee on Secondary European Legislation last week Mr Callaghan's junior Minister, Roy Hattersley, left no room for doubt that UK negotiations with her European partners would not be aimed at achieving such a broad exclusive zone within the proposed 200-mile community strip. Neither Mr Hattersley nor the junior minister at Agriculture, Fisheries and Food, Ted Bishop, felt compelled to comment on speculation that the UK will press for a 35-mile national limit.

What is certain is that Britain will not use her veto to reject the Commission's proposals. Mr Hattersley told the committee that the real problem was rather whether her partners could be dissuaded from using their vetoes to block British pressure for an adapted Common Fisheries Policy. The British view is that adaptation is crucial because, while apparently hamstrung over exclusive limits by obligations under the Treaty of Rome, her industry feels that it will lose more than other EEC members if the Commission's call for national quotas based on past catches is adopted. The practical uncertainties surrounding the effective policing of an eventual agreement add to the complications.

● The Secretary of State for Energy, Anthony Wedgwood Benn, who last week withdrew from the battle for the Labour Party leadership, is continuing his fight to introduce a long-term energy strategy for Britain. He now intends to approach the issues in Britain's energy policy on a wider front, and plans are under way for a meeting in June or July, when it is hoped that several major international oil companies will line up with management and union representatives from the newly-formed British National Oil Corporation and the other nationalised energy industries. ●

INDIA

Becoming well oiled

India is increasing its investment in oil, and the aim is self-sufficiency. Our correspondent in Jullundur reports on a vital aspect of the sub-continent's energy equation

For over two years now, India has been groaning under the weight of her mounting oil-import bill. From around 3,000 million rupees a year before the 1973 Arab-Israeli war it is now running close to 12,000 million rupees—accounting for a third of her entire export earnings and a quarter of the total value of her imports. This is despite the fact that the Government, through a number of fiscal and other measures taken in early 1974, has managed to limit consumption and to curb the rate of growth of demand for oil. These measures remain in force.

Consumption rose in 1975 by only 2.7%, from 21.74 million tonnes to 22.34 million tonnes, the 1974 figure being substantially lower than an estimated 25 million tonnes calculated on the basis of an earlier average annual growth rate of about 9%. Consumption of petrol during 1975, at 1.25 million tonnes, was slightly lower than the 1974 figure of 1.28 million tonnes, despite a considerable increase in the number of powered vehicles in the country; the items contributing to increased consumption of petroleum (and products) included naphtha (14.5%), kerosene (5.5%), high-speed diesel (4.7%), furnace oil (0.5%) and cooking gas (14.3%). The increase in the offtake of naphtha was largely due to better capacity utilisation by fertiliser manufacturing units, while good rains helped dampen demand for diesel for agricultural purposes which was running some 12% higher during the first six months of 1975 compared to the same period in 1974.

India has been meeting only about a third of her oil requirements (including related products) from indigenous sources—mainly onshore wells in the states of Assam and Gujarat. She has had to import the rest from various oil-exporting Arab countries and Iran through bilateral agreements. The Union Petroleum and Chemicals Minister, Shri K. D. Malaviya, recently told the Parliamentary Consultative Committee attached to his Ministry that, because of India's adverse balance-of-payments and foreign exchange positions, the situation regarding imported oil was likely to be very difficult during 1976. Neither was it known how and when OPEC's oil facility for developing countries would be set up, nor when results would start flowing from the ongoing "north–south" conference. The country expected to produce 9.5 million tonnes of oil this year, Shri Malaviya told the committee, and his ministry was making all possible efforts to ensure an import of 14 million tonnes from external sources to meet the country's essential requirements. [It has since been reported that arrangements have now been made for the import of a major part of the crude oil India needs from other countries].

India has passed through a very difficult period but, while she is still not completely out of the woods, pressure seems to have eased somewhat due to an all-round improvement on the energy front. Among other things, coal production has increased enough to wipe out scarcities, and power generation is presently pretty close to meeting all requirements so that power cuts imposed in most states have been withdrawn. While much of the improvement has come through better management and better performance by both man and machine, good and plentiful rains during 1975 have played no mean part in transforming the picture.

For all the havoc it has wrought on the country's economy, the oil crisis has also done a few good things: the Oil and Natural Gas Commission (ONGC) has been stirred into vigorous action and its redoubled efforts to find more oil onshore and offshore are beginning to yield results. From onshore fields in Assam and Gujarat, production this year is expected to be more than 8.2 million tonnes compared to 7.5 million last year. In three to four years' time, according to the Petroleum and Chemicals Minister, the figure will go up to 11 million tonnes.

Production could commence shortly from India's first offshore wells in the Bombay High region. Two developmental wells, along with other installations including submarine pipelines to take oil to the shore, are nearing completion. Production will start at the rate of 2 million tonnes a year by the end of 1976, and will gradually be stepped up to 10 million tonnes by the end of the decade. While the ONGC explores and exploits the Bombay basin, two foreign firms are working on the Bengal and Kutch basins. The very first well drilled in the Bay of Bengal was reported to be oil bearing, raising hopes of further breakthroughs. [The Minister recently announced an oil discovery of "very great significance" at the Bassein structure off the western coast].

In 1974, the ONGC acquired three new vessels, two for drilling in the Bombay High region and a third for seismic surveys in the continental shelf near Madras. The Petroleum Ministry has also set up a "deeper continental shelf" project to explore the 20,000 km region west and north-west of the Bombay High area. Various steps are also being taken to make the country self-sufficient in the manufacture of all types of equipment needed for onshore and offshore exploration and production of oil. The first country-made rig is expected to be available in about two years' time. A variety of oil-field equipment is already being manufactured in India, and its production is being stepped up.

India has also entered into agreements with Iran and Iraq to look for oil in these countries. According to the Petroleum Minister, some 750,000 tonnes of Rustum crude from India's exploration venture in Iran will be brought to India this year.

Before the turn of the century, India is expected to become self-sufficient in crude oil. Some optimists think she could even become an exporter of oil by the year 2000. ☐

Science and religion

If circulation figures of periodicals and journals are any indication, religious subjects continue to score over scientific ones in popularity among the literate population in India, which has the world's third largest scientific and technological community. According to a recent study, there were 927 scientific and technical journals (all languages) compared to 1,221 religious and philosophical ones in the country by the end of 1973, and circulations of the latter went up by more than the former over the decade 1963–1973.

Broken down by language, the figures for the circulation of Bengali, Tamil, Kannada and Gujarati science periodicals showed a downward trend during the decade, whereas those of the religious journals in these languages went up over the same period. Marathi, the language of the state of Maharashtra, had the unique distinction of having more readers of scientific journals than those of the religious ones. Urdu was found to be another language with a distinction—it showed an increase in readership of scientific journals with a simultaneous decrease in circulation of the religious periodicals. In English and Hindi—the two languages with the largest proportion of periodicals on all subjects—readers seemed to prefer religion to science.

IN BRIEF

Group of Thirteen?

The seven nuclear powers who recently agreed terms extending safeguards against the misuse of exported nuclear technology are expected to be joined in their pact by six more nations, reportedly after Soviet pressure to have more eastern bloc countries admitted. Belgium, Czechoslovakia, East Germany, Poland, Sweden and the Netherlands would participate with Britain, Canada, France, Japan, the USSR, the USA and West Germany in an agreement which allows nuclear trade with states outside the Non-Proliferation Treaty. The report follows news that France is planning to sell nuclear reactors to Libya, a major oil producer facing none of the problems of energy shortage.

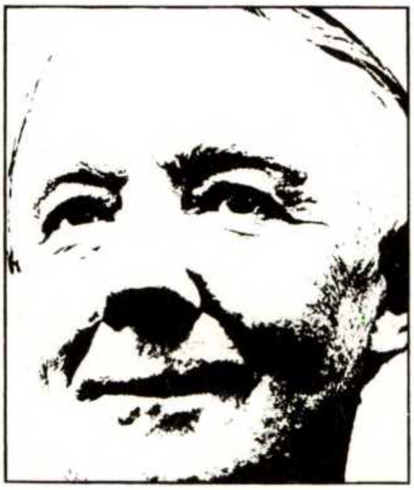
LST refocus

The US House of Representatives last week took the first step toward rescuing the Large Space Telescope (LST) from acute budgetary failure, by passing a bill which authorises the expenditure of $3 million on planning and instrument design for the mission. At the same time, however, the Senate Aeronautical and Space Science Committee approved a bill which, in tune with the Ford Administration's own decision for the coming fiscal year, contains no funds at all for the LST. But the LST seems very likely to get full backing next year.

Shtern support

The case of Dr Mikhail Shtern, the Ukrainian endocrinologist at present serving an 8-year-sentence in a labour camp near Khar'kov, continues to evoke support. On March 24 a petition was published in Paris, signed by 51 Nobel Prize winners, accusing the Soviet government of fabricating charges against him and demanding his release. Ironically, the same day, Dr Shtern, who had made an appeal for clemency on the prompting of the camp authorities that it would be favourably received, was informed that it had, in fact, been rejected.

A hundred years on



KENNETH MELLANBY

The year 1976 is the hundredth anniversary of the passing of the Cruelty to Animals Act, which still controls the way in which painful animal experimentation — "vivisection" — is performed in Britain. There is now considerable pressure to introduce new legislation, partly because of a general feeling that a law made over a hundred years ago must, in some ways, be out of date. Animal experiments have indeed changed during that period. In 1876 only a few hundred experiments were performed each year, and a high proportion involved surgery. Today we make some five million experiments per annum; the majority are to test drugs, pesticides, food additives, cosmetics and other chemicals, and consist of feeding trials which may involve little of the type of "pain" with which the original Act was concerned.

The 1876 Act is unpopular with lawyers, perhaps because it has given rise to so little litigation. It would be cynical to suggest that the lawyers' criticism stems from the fact that so few fees have been earned in this field; their more legitimate complaint is that the absence of decisions by the courts may leave the interpretation of some of the Act's provisions ambiguous. However, the exhaustive deliberations of the Royal Commission set up in 1906, and contained in their report which eventually appeared in 1912, suggested that radical changes were not required. The very thorough report, in 1965, by the Home Office's Departmental Committee on Experiments on Animals (the "Littlewood" report) came roughly to the same conclusion. The Act may not be technically perfect, but it works better than many newer pieces of legislation. Once more there is much to be said for leaving well alone, though the Littlewood proposal for a more effective Advisory Committee, containing lay members, would seem worth implementing without further delay, notwithstanding the apparent opposition of the Home Office.

In my experience most responsible experimenters have a strong feeling that certain potentially painful experiments are seldom if ever justified. This is an ethical judgement, but it may have a scientific basis. Some countries have no regulations to prevent any cruel experiment, and even some states in the USA allow practices outlawed elsewhere. Thus we read of school children inserting electrodes in monkey's brains, and I recently reviewed an American book in which children were instructed how to subject rats and other mammals to noise intensities sufficient to destroy their auditory tissues. It is sometimes argued that the controls imposed by the 1876 Act hold up medical and scientific progress. If this were so, then we would expect striking advances from the regions where ethical controls do not exist. In fact progress has generally been greatest in the laboratories where controls are most strictly enforced.

Some anti-vivisectionists insist that animals are so different from man that experiments on animals are generally inapplicable to human conditions. It is true that, in the end, new drugs and treatments must be tried out on man himself, but without preliminary animal experiments on several species most physicians would be even more reluctant than at present to make these trials. More experiments on man would probably mean doing more, not fewer, animal experiments. In fact there are already many instances where "human guinea pigs", healthy volunteers, are usefully employed, though in a civilised country, except for the often-gruesome experiments done by some scientists on their own bodies, this type of work has been considerably restricted. It is sometimes suggested that important medical problems could be rapidly solved if humans were used without any ethical constraints. This I doubt. We have the experience gained from experiments on prisoners in Nazi concentration camps. Here humanitarian concern was seldom allowed to modify the plans of the scientists, and millions of victims were killed in the name of research. Yet no major discoveries were made. It seems that the brutal scientist is generally a bad scientist, and it matters little whether he works with animals or with man.

If we are to have new legislation, research workers as a whole would welcome it if it only curbed the excesses of the minority of bad and cruel workers, but there is a danger that it might make much productive and valuable work, done under good ethical conditions, impossible.

correspondence

International transfers

SIR,—The following document is communicated for publication on behalf of the 52 Fellows of the Royal Society whose signatures appear below. No doubt other members of the scientific community in this country and throughout the world will wish to affirm their support for the same principles. Those who share our concern about these matters may wish to know that a Study Group on Scholarly Freedom and Human Rights has been established by the Council for Science and Society (3/4 St Andrews Hill, London EC4V 5BY) to give further consideration to the issues involved. It would be particularly valuable to the work of the Study Group to have further detailed information or well informed opinion from any of your readers.

Yours faithfully,

J. M. Ziman

and M. F. Atiyah, C. H. Andrewes, C. Auerbach, J. F. Adams, J. G. Bolton, J. Baddiley, K. G. Budden, C. A. Clarke, S. Curran, R. E. Davies, F. Dainton, C. D. Darlington, K. G. Denbigh, L. Essen, C. A. Fleming, G. Fryer, G. Herzberg, W. Hudson, A. Haddow (deceased), J. T. Houghton, J. H. Humphrey, W. K. Hayman, A. Klug, A. Kelly, H. Kornberg, O. A. Kerensky, B. Lockspeiser, L. F. La Cour, H. Lipson, J. D. McGee, E. H. Mansfield, J. Mandelstam, K. Mahler, A. Neuberger, J. Needham, L. E. Orgel, A. G. Ogston, M. R. Pollock, J. W. Pringle, W. S. Peart, G. Rochester, A. Robertson, R. A. Raphael, N. Sheppard, G. Series, M. Seaton, M. Swann, M. Szwarc, R. Whittam, M. Wilkins, F. Young.

The following statement is proposed for consideration and adoption by learned societies, academies, governmental bodies and private corporations involved in the practical arrangements for the international transfer of scientific information by written communication and by personal travel. Although not intended to be legally binding, this statement indicates the basic principles by which we consider that particular agreements and actions in this sphere ought to be judged.

International Scientific Communication

The claim of scientific knowledge to universal validity can only be tested and maintained by international intercourse between scientists. All nations which make use of scientific knowledge benefit by the rapid diffusion of information about new discoveries, and by the submission of new ideas to the widest circle of competent critics. The free exchange of knowledge and ideas is fundamental to the health and vitality of science as an enterprise of all mankind.

The individuals and corporate bodies who benefit directly from this intercourse should be bound by certain common courtesies and mutual obligations. Although these courtesies and obligations are normally taken for granted, their neglect or violation can cause much ill will and damage to science as a whole. We therefore believe that there is value in stating the following general principles, which are based upon the experience of communities in which science has flourished in many different historical and political circumstances.

A. Written Communications

1. The published literature of science should be free to circulate, without hindrance or censorship, within and between nations.

2. All efforts should be made to ensure that relevant scientific information published in any one country be made available, as rapidly as possible, in the most convenient form, to scientists and to scientific institutions in all other countries.

3. The originality of published scientific work should be respected throughout the world by fair practices of citation or other attribution.

4. The legal rights of authors and publishers of scientific journals and books should be protected under the International Copyright Agreement.

5. All national scientific communities should contribute to the financial support and management of international facilities for the transfer of scientific information such as abstracting and indexing services.

B. Individual Travel

1. Scientists acknowledged by their colleagues as experts should be free to travel within and between different countries for the exchange of unclassified scientific information.

2. Acknowledged scientific experts making such visits should be given intellectual hospitality in the form of access to open research institutions and to individual scholars.

3. The services of scientific experts from all countries should be made available to assist in the activities of the international scientific agencies.

4. Bilateral financial and administrative arrangements for the exchange of scientists between two countries should not be regarded as excluding or limiting visits arranged by other means.

C. Conferences

1. The appropriate national scientific organisations should be encouraged to participate in open international scientific conferences by facilitating the attendance of well-qualified scientific experts in the relevant subjects.

2. All countries should endeavour to contribute to the material facilities for international scientific conferences by way of hospitality and financial support.

3. Every bona fide participant in an international scientific conference should be accorded freedom of entry to the meeting place of that conference.

4. The expert authority of properly constituted international committees of scientists should be respected in such matters as the choice of participants and speakers at international scientific conferences.

Genetics at the OU

SIR,—The article by Professor Steven Rose on the new genetics course offered to Open University students (*Nature*, **259**, 437; 1976) is misleading. In particular, it does not refer to the existence of a Consultative Committee, of which we are members, appointed by the Nuffield Foundation to advise on the development of the course, and to the sharp disagreement between that committee and Professor Rose about the suitability of the materials now being distributed for use by students at large but also by Open University students.

The Joint University Genetics Course was launched in 1973 with a grant of £20,000 from the Nuffield Foundation with the objective not merely of developing an outstanding course in genetics but also of proving a mechanism by which the Open University and other universities might collaborate in the development of educational materials. At the same time the Foundation appointed a Consultative Committee to advise the course team on academic matters and to advise the Foundation on the progress of the work. Apart from members of the course team, the committee included Professor D. Lewis (University College, London) as chairman of the committee, Professor W. Bodmer (University of Oxford), Professor J. Jinks (University of Birmingham), Professor D. Jones (University of Hull), Dr H. Kacser (University of Edinburgh), Professor R. H. Pritchard (University of Leicester) and Professor J. Sang (University of Sussex). In March 1974 the total amount of the grant from the Nuffield Foundation was increased to £88,000.

At the end of 1975, nearly three months before Professor Rose's article appeared, the committee informed the Nuffield Foundation that it was dissatisfied with the outcome of the previous three years' work and asked that the course should not be published, or even used by Open University students, without further consideration, restructuring and amendment. The Nuffield Foundation has informed the Open University of the committee's discontent but no agreement has yet been reached between the Foundation and the University.

The length and depth of the course was thought to be far beyond the capacities of the intended students. The Open University states that the study time for the average "unit" is 12 hours. Furthermore, these 12 hours must be completed, on the average, in one week since the course is a half-credit. This, for a part-time student, means a minimum of two hours daily study after normal work for the whole of the academic year. It is also a *second* level half-course. It was the committee's considered opinion that this is an impossible task if understanding at a university level is aimed at. In their experience, the course, as it stands, would be a demanding one for full-time students having no second half-credit to cope with and enjoying much greater academic support than Open University students. The committee suggested that the university should cut the course in half or offer two alternative (half-) courses.

In spite of the original remit to write a course which is suitable for other than Open University students, the Consultative Committee has not been presented with any evidence that this problem has been seriously considered. One of the principal claims for obtaining support from the Nuffield Foundation was that it was not a purely Open University exercise but that other institutions would benefit equally. It is extremely doubtful if this objective is in any way near realisation. (One of the obvious deficiencies of the course soon to be offered for general sale is that the practical manual that students at universities other than the Open University will need has not been published, or even written.)

The Consultative Committee jointly with the Nuffield Foundation suggested a number of changes which would have gone some way to meet these criticisms, but they were not accepted by Professor Rose.

It is not appropriate to comment at length about the many doubts and criticisms which the committee had both about the nature of some of the units and the manner in which the course was produced. Suffice it to say that it is the opinion of the committee that the adherence to a timetable, set down before the magnitude of the problem was appreciated, made it almost mandatory that no fundamental thinking could inform the writing of the course. We have sadly come to the conclusion that a great opportunity has been lost in making use of the generous provision of the Nuffield Foundation.

Yours faithfully,

H. Kacser
Department of Genetics,
University of Edinburgh
R. H. Pritchard
School of Biological Sciences,
University of Leicester
J. Sano
School of Biological Sciences,
University of Sussex, UK

Birds on the Chagos Bank

Sir,—The Great Chagos Bank, 6°15′S, 72°E is all that remains of a once gigantic atoll. The dry land now consists of eight small islands set on the rim of the atoll and an outlying group of six other islands collectively called Egmont. Together they constitute a terrestrial habitat of only 828 ha, of which Eagle and Egmont Islands make up 80%.

Records indicate that at some time between 1813 and 1937 all the islands came under the influence of the copra industry. Only Nelson island was left in its "natural" state which included colonies of nesting sea birds, the eggs of which were collected to supplement the diet of the copra workers on the nearby Salamon group.

Recent surveys show that:

- on all the islands broad-leaved forest is beginning to replace the coconut plantations;
- on all islands other than Eagle and Egmont there are large populations of nesting sea-birds, numbering in total more than 100,000 pairs—dominantly terns and boobies, but including 15 species in all;
- on Eagle and Egmont there are feral brown rats and only a few hundred birds; the vegetation is, however, similar to that of the other islands.

In order to realise the full potential of the island group to nesting seabirds we suggest the removal of the rats from Egmont and Eagle Islands using a combination of methods such as "warfarin" and Liverpool virus. The islands are remote from any large centre of population and are rarely visited. Such action could allow the already overcrowded bird colonies on the local islands to expand and new species to move in from further afield. For instance, pairs of Abbots Booby were seen in the vicinity of the islands on two occasions. This is the rarest of the Indian Ocean's gannets, whose only contemporary nest site on Christmas Island is threatened by development and ecological change.

We estimate that if such action were successful the total nesting population of birds in the area could rise to in excess of 0.5 million pairs.

We are planning, subject to legislative approval, to attempt to exterminate the rats on Egmont and Eagle Islands in early 1977. We invite comment on the desirability and feasibility of this course of action.

Yours faithfully,

M. J. Hirons
College of Education,
Easthampstead Park, Berkshire
D. J. Bellamy
C. Sheppard
Department of Botany,
University of Durham, UK

Bugs Bunny

Sir,—Schedule 1 of the Conservation of Wild Creatures and Plants Act 1975, passed recently by the UK Parliament, provides the best way of giving full protection to any animal whose survival is threatened. To be included an animal must be given a common and a scientific name ("Naming the Loch Ness Monster", *Nature,* **258,** 406; 1975). Thus, after ten years of searching, we would like to present the evidence on which we base our description and naming of the "Model E Rabbit".

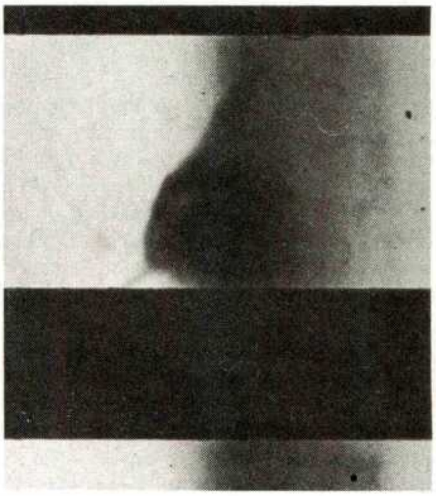
The best photograph shows the head of the rabbit obscuring the DNA bands of an analytical caesium chloride gradient (below). This gradient was run in a Beckman Model E Analytical Ultracentrifuge (Wells, R., and Ingle, J., *Pl. Physiol., Lancaster,* **46,** 178–182; 1970), and the rabbit must have obscured the light path while the plate was exposed.

We feel that previous failure to observe these rabbits, for surely there must be a breeding population, is due to their shy and retiring nature; the DNA on this occasion was from cucumber hypocotyls and their inordinate fondness for these may have led to this rash exposure.

In the interest of protecting this rare and endangered species we have decided to name it *Oryctolagus barbifugenter* because of the curious and distinctive beard-like structure characteristic of the species and because the animal so readily flees from view. It has been pointed out to us that this binomial is an anagram of "Centrifuge laboratory bugs", but this is thought to be entirely coincidental. We hope for the support of the World Wildlife Fund in our efforts to preserve and study this unique rodent.

Yours faithfully,

M. J. Pascoe
L. Potts
Department of Botany,
University of Edinburgh,
Edinburgh, UK



news and views

How far does pollen travel?

from Peter D. Moore

POLLEN grains contain the male gametophyte generation of flowering plants. They are rendered mobile by such forces as wind, water or insect vectors, and the distances which they travel determine to a large extent the degree of gene movement between populations of a species. It is therefore of considerable interest to a geneticist to establish how far pollen of a particular species is dispersed. Such a knowledge is also of value to the palaeoecologist, who is concerned with the interpretation of fossil assemblages of pollen grains in terms of the vegetation which gave rise to them; reconstruction of past vegetation can be considerably aided by information regarding the dispersal potential of different species.

The distances moved by pollen grains depend on their density and aerodynamic properties, which vary from one species to another. Erdtman (*Handbook of Palynology*, Munksgaard, Copenhagen; 1969) has estimated the settling velocities of various types of pollen grains from the air and has obtained such values as $3\ \mathrm{cm\ s^{-1}}$ for *Pinus*, $1.6\ \mathrm{cm\ s^{-1}}$ for *Alnus* and $0.9\ \mathrm{cm\ s^{-1}}$ for *Juniperus*. These three species are wind pollinated and one might suppose that a low settling velocity will be accompanied by a high proportion of the grains produced by a plant being transported over long distances. Precise correlation studies are complicated by the fact that some species produce far more pollen per individual than do others. The local surroundings of the pollen-producing plant will also have an important influence both upon the amount of pollen produced and upon the distance which it is likely to travel. The hazel, *Corylus avellana* for example, flowers far more abundantly on forest edges and in clearings than it does when enclosed by a woodland canopy. Tauber (*Proc. Congr. Int. Assoc. Quatern. Res.*, **7**, 131; 1967) has also demonstrated that plants surrounding the pollen-producing individual act as a filter. Pollen grains become impacted upon solid obstacles in just the way that snow adheres to tree-trunks and hence their dispersal potential is reduced. This filter acts in a differential manner, for large grains are more easily impacted than small ones. The height above the ground at which the pollen is released will also influence the distance over which it is likely to remain airborne.

In the case of wind-pollinated species, the atmospheric turbulence patterns are also of crucial importance in determining dispersal and Tauber (*Danm. geol. Unders.* **IIR 89**, 1; 1965) has devised a detailed model of pollen transport above and below woodland canopies, which takes into account the low wind speeds found within tree canopies and the slightly raised wind speeds within the trunk space. Some canopy-produced pollen will be incorporated into air masses moving at higher speeds above the woodland shelter and this may be carried considerable distances. Tyldesley (*New Phytol.*, **72**, 175; 1973) has recorded the pollen of trees, mainly birch and pine, on the Shetland Islands in densities of up to 30 grains $\mathrm{m^{-3}}$ of air. The movement of air masses before collection suggests that these could have been transported from Scandinavia. Similarly, Moar (*New Zealand J. Bot.*, **7**, 424; 1969) has reported the pollen of *Casuarina* in surface samples from New Zealand, when the nearest plants of this genus grow in Australia. On the basis of aerial transport studies on pollen in Canada, Christie and Ritchie (*Naturaliste Canad.*, **96**, 531; 1969) come to the conclusion that the mean life of pollen grains in the troposphere is about three days.

It is difficult to assess the importance of such long-distance pollen transport mechanisms to the process of gene flow. Since only a very small proportion of pollen grains produced is likely to be carried very far, one assumes that these grains are of little functional significance. Yet Mason (in *Genetics, Palaeontology and Evolution* (edit. by Jepsen, Simpson and Mehr), 356, New York; 1963) records the invasion of *Pinus muricata* genes into populations of *P. remorata* on the islands of Santa Cruz, over 30 miles offshore. This must have come about as a result of pollen movement from the mainland, hence long-distance pollen transport can be an important vehicle for gene movement.

The study of pollen movement has been rendered difficult by the problem of relating pollen grains, once dispersed, to the producer individual. The process of gene movement itself has proved a useful indicator of pollen dispersion, for example in the studies of rye grass, *Lolium perenne*, by Griffiths (*J. Agric. Sci., Camb.*, **40**, 19; 1950). Such studies have shown that wind-pollinated species have a pollen dispersion pattern which is leptokurtic; this distribution pattern resembles the normal distribution, but frequencies fall more rapidly on either side of the mode (see Proctor and Yeo, *The Pollination of Flowers*, 262, Collins, London; 1973). Wright (*J. Forestry*, **51**, 114; 1953) has expressed the dispersal capacity of certain trees in terms of the standard deviation of their leptokurtic pollen curves.

The use of radioactive labels on pollen grains has been attempted. Colwell (*Am. J. Bot.*, **38**, 511; 1951) tagged the pollen of *Pinus coutteri* with $^{32}\mathrm{P}$ and collected dispersed pollen in open Petri dishes for counting. He showed that the bulk of the pollen released was deposited within 10 m. Chamberlain and Chadwick (*Ann. appl. Biol.*, **71**, 141; 1972) have applied tracer techniques to the study of pollen impaction on solid objects. The usefulness of such techniques for the study of pollen dispersal in the field, however, is limited by the environmental hazards involved.

In this issue of *Nature* (page 422), Handel describes the use of neutron activation analysis in an experiment to determine the extent of pollen dispersal from two species of *Carex* in a woodland habitat. A solution of samarium oxide was injected into male spikelets and the day after pollen was shed, female spikelets from the same and from neighbouring plants were collected. When samarium is bombarded with neutrons it is converted into europium, ^{153}Eu, which emits gamma rays which can be counted. In this way, samarium-containing pollen can be located accurately. The results obtained obey the characteristic leptokurtic distribution, and show that very little pollen of either species tested is carried beyond 1 m, although the species with the taller culm (20 cm as opposed to 12 cm) does have better powers of dispersal. The very small distance travelled by the pollen of these understorey herbs confirms what has been assumed about pollen movement beneath tree canopies. It also suggests that there must be a limited flow of genetic material between isolated individuals or populations of such species in woodlands, even if they produce copious pollen, depend upon wind pollination and have a tall culm.

The technique of neutron activation analysis in pollen studies offers considerable opportunities. The field is now open for the study of pollen movement from canopy species, the precise influence on dispersal of impaction on tree trunks, pollen dispersal from clearings and woodland edges and also the movement of pollen in insect-pollinated species. The technique, given the basic equipment, is fast and accurate and lacks the environmental hazards associated with radioactive labels. We can now await a flow of new data which will be of value to geneticist and palaeoecologist alike. □

Hominid remains from Hadar, Ethiopia

from our Palaeoanthropology Correspondent

ALTHOUGH fossil-bearing deposits in the Hadar region of eastern Ethiopia have been explored only since 1973 it is already clear that this area is of extraordinary importance. As two recent papers indicate (*Nature*, **260**, 289, 293; 1976), extremely favourable depositional conditions have allowed the preservation of many fossil specimens, even crocodile and turtle eggs, in a virtually complete state. This is an almost unique situation for Plio–Pleistocene deposits in Africa. Moreover, appropriate geological materials are available for chronometric dating of the sequences and although the absolute dates obtained so far are not entirely satisfactory, this problem should soon be resolved. Available information indicates that the hominid sites in the Hadar area are between 2 and 4 million years.

As reported in last week's issue, fossil material from approximately 17 hominids has been recovered from sites in the Hadar region. Of these, one of the most significant is AL 288, a partial skeleton recovered from the younger levels of the sequence. The partial skeleton is of a very small hominid which bears many similarities to the australopithecines from Sterkfontein, in South Africa. Certainly one of the most interesting aspects of this individual, from information available so far, is the direct indication of limb proportions in this early Pleistocene hominid. Estimates of upper and lower limb proportions have been made previously but never on virtually complete bones from a single individual. The ratio of humeral/femoral length in AL 288 is 83.9; living *Homo sapiens* has a mean of approximately 73. By comparison, among the living great apes *Pan*=102, *Gorilla*=116, *Hylobates* =113 and *Pongo*=135. (Schultz, *Hum. Biol.*, **9**, 1937). Thus, although the length ratio of AL 288 is somewhat above the human mean it is distinctly lower than that for the living great apes. Such a relationship has been expected, especially in view of the long humerus from East Rudolf (Leakey, Mungai and Walker, *Am. J. Phys. Anthrop.*, **36**, 1972) and the long ulna from the Omo River area just to the west of Hadar (Howell and Wood, *Nature*, **249**, 174; 1974). These data clearly indicate the essential man-like body conformation of AL 288. This is of particular interest in view of Oxnard's recent claim (*Nature*, **258**, 389; 1975) of the orang-like morphology of the australopithecines. This new information on limb proportions, along with the clear evidence of erect bipedality in the morphology of the pelvis, femur and knee joint does not lend support to such an assertion. Moreover, it indicates that computer-oriented analyses on single bones, however sophisticated the statistics, may be of somewhat limited value in determining morphological and functional affinities of fossil material. This is especially true when, as Oxnard recognised, the functional pattern of the fossil specimen may have no modern parallel.

Of additional interest in these papers is the report of two palates (AL 199 and AL 200) which Johanson and Taieb claim resemble other material referred to as *Homo erectus*. This material is from the lowest stratigraphic unit known to date in the Hadar region; if the data on the Kadada Moumou basalt is confirmed then this material would pre-date 3 million years. This possible early occurrence of *Homo erectus* is of particular interest since the re-dating of the KBS tuff in the East Rudolf area (Curtis *et al.*, *Nature*, **258**, 395; 1975) would suggest that the early members of the genus *Homo* in that area are little more than 2 myr old.

Results of field work in the past few years have made it clear that at least two forms of hominid co-existed in east and possibly South Africa in Plio–Pleistocene times: one, a less advanced form usually called *Australopithecus* (or *Paranthropus*) and a more advanced form, *Homo*. This picture has achieved additional confirmation from the new material recovered at Hadar. The complexity of the fossil evidence at East Rudolf has led Richard Leakey to suggest a possible total of four hominid lineages there (*Nature*, **248**, 653; 1974) and Johanson and Taieb, in the present paper, suggest possibly three at Hadar. Whatever the total number of contemporaneous hominid taxa in this area, it is clear that we are not seeing a simple and orderly progression of ancestor–descendant populations. The most economical interpretation of this diversity is that the early hominid line, once it had come to occupy the forest-margin/savannah ecozones, experienced a number of radiations and adaptations to this habitat. Such adaptive radiations are common during the initial occupation of a new habitat zone and serve to fit the immigrant populations to the available new niches. The molecular evolutionists, who have postulated a relatively recent origin for the family Hominidae, have made palaeontologists somewhat reluctant to talk about a great time depth for the hominids. But that is exactly what this Plio–Pleistocene diversity indicates. Such diversity, with two or more lineages, clearly suggests considerable antiquity for the human line, with splits having occurred in different places, at different times and in response to different environmental situations. Perhaps the appropriate question, however, in the face of such apparent diversity is not how many hominid lineages have existed but how many hominid econiches are available in such a habitat? □

Sociobiology: a new synthesis and an old quarrel

from Robert M. May

One of the most notable books published last year was *Sociobiology: The New Synthesis* (Belknap, Cambridge, Massachusetts; 1975), by Edward O. Wilson. This massive work aims to draw together a growing body of field and laboratory observations on animal behaviour, and combine it with insights drawn from population biology and ecology, to yield an understanding of how different social systems have evolved. Sociobiology is Wilson's term for this systematic quest for an evolutionary explanation for all forms of social behaviour. Both explicitly and implicitly the book calls attention to some of the major unsolved problems: this is indeed Wilson's main aim, which would be described more accurately if the title read *Towards a New Synthesis* rather than *The New Synthesis*.

Evolution of social systems

The fundamental principle guiding this sociobiological enterprise is that an organism's evolutionary success is measured by the extent to which its genes are represented in the next generation. One road to such success is for the individual to evolve characteristics (strength, speed, camouflage, a well advertised nasty taste or the misleading mimicry of something else's advertisement) which help it survive long enough to reproduce; these relatively straightforward considerations have provided the focus for most investigations of evolutionary ecology. For many animals there are, however, advantages (such as greater foraging efficiency, or defence against predators) in living groups. Once this happens, the social structure of the community, and the behaviour of individuals within it, themselves become subject to selection. Social systems thus evolve to maximise the genetic fitness of individuals in specific environments.

If the social system is sufficiently close-knit, an individual may enhance its genetic contribution to the next generation by the apparently altruistic act of sacrificing itself in order to help its kin. An extreme example is provided by the sterile worker castes in insect societies; here recent work by Hamilton and by Trivers and Hare has produced quantitative insight into the organisation and evolution of the social insects (see *Nature*, **260**, 9; 1976).

The behaviour of birds and mammals is less stereotyped than that of insects, and elucidating the evolutionary basis of their social structure is correspondingly a more complicated and ambiguous task. Nevertheless, with the growing tendency towards long-term, analytical field studies of individual animals whose consanguinities and social histories are recorded, much progress is being made.

Consider, for example, the studies by Schaller, Bertram and others on the Serengeti lion (for example Bertram, *Sci. Amer.*, **232**, (5), 54–65; 1975). Roughly speaking, the typical female lion spends her life as a member of the pride into which she is born, and may live about 18 years. In contrast, male lions leave or are ejected from the pride at puberty, and they will usually enjoy a later brief tenure of 2–3 years, typically along with one or more brothers, as the only adult males in another pride: the more brothers sharing the rule, the longer the tenure. It seems that, soon after taking over a pride, the new males kill the resident cubs. Since the females tend not to produce a new litter until the previous cubs are 20–30 months old (which time is of the same order as the males' expected tenure as owners of the pride), and since the new males usually share no genes with the pre-existing cubs, the males' inaugural act of infanticide is adaptive from their point of view, although it is certainly not in the females' best interest.

This is a particularly brutal example of the tensions between male and female interests which arise in diploid systems when parental care is important, as it is among birds and mammals. A female's genes are represented directly only in the offspring she herself produces, and so it tends to be in her interest to care for them (at least until they begin to be able to look after themselves, whereupon at "weaning" a parent–offspring tension arises because it is in the child's interest to continue to enjoy parental care, whereas the mother will do better to turn attention to producing a new litter). A male's genes have a chance of direct representation in the progeny of every female he inseminates, and in some circumstances his genetic input into the next generation will be maximised by striving for copulations with many females (even at the expense of evolving otherwise maladaptive ornaments, and at the risk of injury in fights with other males), and giving no help in raising the children. In short, in many situations there is a built-in tendency for females to focus on ecological resources such as food and nesting sites, and for males to focus on females.

Mating systems

These notions, when combined with the ecological details of particular environments, help explain the bewildering diversity of mating systems observed in bird and mammal societies: monogamy; polygamy (either as polygyny, one male with several wives, or as polyandry, one female with several husbands); promiscuity. In some situations raising offspring is necessarily a two-person job, leading to monogamy. Monogamy is the rule among birds (92% of all bird species are monogamous), and is uncommon among mammals, where the mother suckles the young. In environments where resources are very unevenly distributed, it may pay a male to devote much energy to defending a good territory, and it may pay a female to share that territory with other wives rather than have exclusive partnership with a male who owns inferior real estate; this leads to polygyny. Polyandry should be very rare, and it is. When the female is capable of feeding both herself and her offspring, as is the case for most herbivorous mammals, we expect a tendency towards promiscuous mating systems. But carnivorous mothers need help: all raptorial birds are monogamous, and carnivorous mammals tend to have complicated mating systems (such as the lions, above).

When the environmental circumstances dictate that a male should opt for monogamy, and devote his time and energy to help rear offspring, it will be adaptive for him to react violently to the possibility of stolen copulations with his wife. In one series of experiments, Barash (University of Washington) placed a model of a male mountain bluebird near the nest, during the breeding season, while the real male bluebird was temporarily absent: on its return, the male fiercely attacked both the model and its own mate, in one case even driving her away and taking a new mate. Once the eggs had been laid, the male was much less traumatised by this mean trick, and did not attack its mate. This experiment, incidentally, shows how the manipulative techniques that shed so much light on the adaptive significance of animal behaviour cannot blithely be applied when dealing with humans.

Sociobiology summarises many other aspects of animal behaviour and social organisation that can be illuminated by evolutionary thinking. A crisp review of some of this work is due to Kolata (*Science*, **187**, 50–51 and 156–157; 1975). When one comes to the primates (excluding man), social structures become very complicated and diverse, ranging from troops with a complex social organisation (baboons), through nuclear families (white-handed gibbons, with a single mated pair accompanied by up to four immature offspring and an occasional aged male), to individuals who spend most of their lives in solitary fashion (male orangutans).

So far I have touched on 25 of the 27 chapters in *Sociobiology*, which deal with non-human animals. This work constitutes a *tour de force* of synoptic scholarship, and has not been challenged.

Human behaviour

In chapters 1 and 27, Wilson speculates upon the insights to be gained by applying sociobiological thinking to human behaviour and human societies. The social insects, for example, show how extreme forms of altruism can evolve by virtue of kin selection in conjunction with inbreeding. In the absence of any constraints, there is a strong selective advantage in inbreeding coupled with recognition and cooperation among kin. But in relatively complex organisms, such as humans, there is a severe constraint, namely the well documented increase in the expression of deleterious genetic traits as heterozygosity is diminished by inbreeding. Wilson poses the question: how are the gains through inclusive fitness and losses through inbreeding depression balanced? In which life history strategies is this balance struck so as to increase the likelihood of more intense inbreeding and social life? In particular, he observes that in human societies (excepting Faulkner's Yoknapatawpha County) the incest taboo is both universal and has the merit of minimising the occurrence of crippling genetic malformations. He suggests that this universal taboo has adaptive origins, rooted in some gene complex.

In suggesting that pervasive human behavioural manifestations such as spite, indoctrinability or courage may have aspects that are genetically controlled, Wilson is at pains to emphasise that he is not implying "that there exists one gene for spite, another for homosexuality, and so on, as one might envisage the inheritance of flower colour or seed texture in garden peas. The tendency to develop such behaviour, in a distinctively human form, is part of an immensely complex social repertory which is undoubtedly dependent on large complexes of genes" (*Harvard Gazette*, January 16, 1976). Given that the relatively simple and stereotyped behaviour of aggregation in slime moulds is under the control of a complex of about 50 genes (Cox, Princeton University, personal communication), I find Wilson's view plausible. It is, however, clear that direct confirmation, in the form of explicitly identifying particular gene complexes for particular behaviour, is out of the question.

These first and last chapters, with their suggestion that human behaviour

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Strong fibres

from Paul Calvert

ONE long-standing objective in polymer science is the production of fibres with an elastic modulus (ratio of stress to strain) approaching the theoretical maximum value. It seems self-evident that the structure should consist of polymer chains aligned as closely as possible to the fibre axis with few folds or disoriented regions. Various methods might produce this structure. The natural and most elegant method is exemplified in the production of plant cellulose where polymerisation occurs *in situ* so that fibres grow by addition of monomer units to one end. This has never really been achieved synthetically.

The drawing process used in the production of textile fibres produces chain orientation by stretching the fibre to several times its initial length. Two fibre industry alumni now in universities have worked on modifying this process to produce high draw ratios and orientation and so high moduli. E. S. Clark of the University of Tennessee and co-workers at Du Pont have produced acetal fibres with a modulus of 35 GPa (1 giga pascal= 10^9 N m^{-2}) (*Polymer Engng Sci.*, **14,** 682; 1974). For comparison, carbon fibre has a modulus of 500 GPa and the theoretical maximum modulus of polyethylene is 220 GPa (Frank, *Proc. R. Soc. Lond.*, **A319,** 127; 1970). These acetal fibres were drawn at about 130 °C by a factor of 20 times in a two-stage process. The usual room temperature draw ratio is 7 times. The structure seems to consist of a single highly oriented phase.

I. M. Ward and co-workers at Leeds have studied the dependence of draw ratio on temperature, molecular weight and pre-draw microstructure in polyethylene. They found that the modulus increases roughly linearly to 70 GPa at a draw ratio of 30 times. The draw ratio was a maximum for a sample of high molecular weight and narrow molecular weight distribution crystallised by cooling to 110 °C then quenching to room temperature and drawn at 75 °C (*Polymer*, **15,** 233; 1974: *Nature phys. Sci.*, **243,** 143; 1973). This leaves the question as to whether it is the molecular weight itself or its influence on the microstructure that is important in allowing a high draw ratio.

Barham and Keller (*J. Materials Sci.*, **11,** 27; 1976) have taken up this point and looked at the relationship between modulus, draw ratio and starting structure. They find that the dependence of modulus on draw ratio is the same for a range of pre-draw microstructures. What did change with microstructure was the maximum draw ratio which could be attained. Sheet which had been directly quenched or made from a sample with no low molecular weight polymer could not be drawn past 20–25 times as opposed to 30–35 times. This point is somewhat confused in the paper by the fact that the figure captions are exchanged.

From this Barham and Keller deduce that the low molecular weight chains, which segregate to the spherulite boundaries during slow crystallisation, govern the drawability of the sample. In support of this they say that samples which draw well had a black line around the spherulites when viewed with crossed polars. Presumably this region is liquid at the drawing temperatures and somehow allows large deformations without fracture. This is surprising in that one would expect strong rather than weak boundaries to be necessary.

Finally, mention should be made of the high melting point, high modulus fibres produced by spinning aromatic polyamides and polyhydrazides from strongly hydrogen bonding solvents such as dimethylacetamide–lithium chloride or sulphuric acid in which they form lyotropic liquid crystals. These fibres spun from anisotropic solutions have moduli several times those of fibres spun from similar isotropic solutions and reach 140 GPa. They are reviewed by Carter and Schenk (*Structure and properties of oriented polymers*, edit. by M. Ward), where the industrial significance is evidenced by the fact that most of the important references are to the patent literature. Two fibres, called Kevlar and Kevlar 49 by Dupont, which are based on poly (p-phenylene terephthalamide) look likely to be used in tyre belting and in composites for body armour and aircraft parts.

may have some components which are genetically determined, have aroused some people's wrath. To suggest for example, that the evolutionary considerations which determine the mating systems of mammals and birds have any light, no matter how oblique, to shed on the tensions and asymmetries commonly observed in human sexual relationships is to invite reflexive dismissal as a "sexist".

A fairly typical example of this criticism is a letter in the *New York Review of Books* (November 13, 1975), written by a group of students and professors in the Boston area, in reply to Waddington's favourable review of the book. This intemperate letter begins by putting the book squarely in a historical tradition which runs from the Social Darwinism of Herbert Spencer, through the gas chambers of Nazi Germany, to E. O. Wilson. It continues with a series of selected and re-arranged quotations which form a collector's item for connoisseurs of polemical literature. The substance of this and other criticisms is that by suggesting a genetic component to human behaviour, Wilson is advocating "biological determinism": they fear that in the hands of political demagogues this can be bent to a defence of the *status quo* as the "natural", and therefore correct order of things.

Broadly speaking, the controversy revives the hoary nature–nurture debate. Wilson is suggesting that human behaviour has innate components, and that there are limits to its plasticity, whereas his critics seem to see man as standing apart from the rest of the animal kingdom, being unique in that human behaviour and social organisation have no discernable genetic elements.

Contrary to the tenor of the criticism, nowhere does Wilson say that human behaviour is totally determined by the genes. He emphasises that there is much about human behaviour that is indeed unique, the product of man's intelligence and social institutions. In an interview with the *New York Times* (November 9, 1975); he said "I see maybe 10% of human behaviour as genetic and 90% environmental. Lewontin [a population geneticist at Harvard who is prominent among the critics] would see it as zero % genetic and all environmental".

This, in turn does violence to the critics' position, which is more subtle. As I see it, their position is rather that until genes for behaviour are specifically identified, the discussion is less than definitive; this being so, and since Wilson's speculations can be perverted to evil political ends, they warrant attack. In Lewontin's words, "At present our ignorance on this question is so enormous, our investigatory techniques so primitive and weak, our theoretical concepts so unformed, that it is unimaginable to me that lasting, serious, truths about human nature are possible. On the other hand the need for the socially powerful to exonerate their institutions of responsibility for the problems they have created is extremely strong. Under these circumstances any investigations into the genetic control of human behaviors is bound to produce a pseudo-science that will inevitably be misused." (*Harvard Gazette*, January 16, 1976).

Wilson's reply in the same Gazette is that "We should be especially cautious about closing off an entire area of investigation when it is in its most vulnerable exploratory stage, because of a generalised fear about its possible eventual misuses in political life. Human sociobiology is in that early stage. I realise that its current hypotheses and facts are susceptible to perversion; equally by ideologues on the right or the left, I might add. We should discourage the perversion—but not the subject."

Shifting from defence to attack, Wilson argues that insofar as human behaviour may contain genetic aspects, social programmes which deny this fact do so to their disadvantage. "[Human] evolution took place under conditions which in many cases no longer exist; our moral precepts, and the economic and social systems that flow from them, will have to be directed to the problems of the future... One does not efficiently eliminate injustice stemming from a genetically based human nature by denying the existence of that nature or prohibiting research on it." He stresses that his views on human sociobiology should not hinder legislation towards social reform, any more than the genetic nature of near-sightedness should discourage people from getting glasses.

The attack on Wilson's book seems to me to have been conducted with a certain incivility, and lack of attention to what the book actually says. When human sociobiology finally comes of age, I hope it will explain why people who are moved to passionate concern for the well-being of Mankind (be it in small disputes such as this, or in grand revolutionary or religious movements) behave so harshly to individual examples of the species. ☐

Disturbing the radiation belts

from Kenneth G. Budden

IT has been known for many years that electromagnetic waves from the Earth's surface can enter the magnetosphere and return to Earth. The earliest known and perhaps the most striking example is the phenomenon of whistlers. These were first observed in the nineteenth century (Preece, *Nature*, **49**, 554; 1894) but were not satisfactorily explained until 1953 (Storey, *Phil. Trans. R. Soc. Lond.*, **A 246**, 113). A whistler originates as the electric impulse from a lightning flash and much of its energy is in the audio frequency range. It travels up through the ionosphere into the magnetosphere and is guided along the lines of force of the Earth's magnetic field, so that it returns to Earth near the magnetically conjugate point in the other hemisphere. During its journey through the magnetoplasma it is dispersed in time so that the high frequencies arrive first. Thus, when received on an aerial connected to an audio amplifier, it is heard as a whistle falling in pitch. Later work showed that signals from other terrestrial sources could travel through the magnetosphere in this "whistler mode". Signals of frequency 15.5 kHz from the US Navy's transmitter NSS at Annapolis, for example, were received at Cape Horn (Helliwell and Gehrels, *Proc. Inst. Radio Eng.*, **46**, 785; 1958) both by the whistler path in the magnetosphere and by the more usual path in the space between the Earth's surface and the ionosphere, which at these frequencies is like a waveguide.

In the magnetospheric plasma these audio frequency whistler waves can only travel in directions making small angles, less than about 19°, with the Earth's magnetic field. This was once thought to be the guiding mechanism but it is not enough to account for the observations. It is now believed that these signals travel in ducts of enhanced ionisation aligned along the magnetic field lines. The signals are confined in the ducts in much the same way as the waves inside a waveguide. But even this is not enough to explain the great strength of some whistler signals. Whistlers have been observed to travel back and forth between the northern and southern hemispheres as many as twenty or more times, and sometimes they get stronger as they travel suggesting that the magnetosphere must be acting as an amplifier.

Since about 1958 it has been known that there are trapped energetic particles in the magnetosphere—the Van Allen radiation belts. These are mainly electrons and protons with energies in a wide range from 10 keV or less to 50 MeV or more, which travel in bent helical paths round the lines of force of the Earth's magnetic field. At the latitudes where the lines of force come down towards the Earth and converge the particles are turned back or "mirrored". Thus they are trapped in stable helical paths with repeated mirroring in alternate hemispheres. A line of force of the Earth's magnetic field is described by its distance from the Earth's centre at the highest point, that is, where it crosses the equator. This distance is written LR_e where R_e is the Earth's radius, and we speak of the L value of a line of force. The radiation belts extend up to about $L = 7$ or 8 with two maxima of intensity around $L \sim 1\frac{1}{2}$ and $L \sim 3\frac{1}{2}$. In between there is a minimum or "slot", in the range $2 < L < 3$, which was shown in Van Allen's first map of the radiation belts (Van Allen and Frank, *Nature*, **183**, 430; 1959).

The particles of the radiation belts can sometimes generate electromagnetic waves of audio frequency. These travel in the whistler mode to the ground and are observed as the remarkable rising and falling whistles that Storey (*op cit.*) called the 'dawn chorus'. The particles can give amplification of whistler waves, operating in a similar way to a travelling wave tube amplifier and this is the amplification needed to explain the observed strengths of some whistlers. Emission of radiation from the particles can be triggered by electromagnetic waves and triggering by whistlers and by signals from radio transmitters of very low frequency has been known for many years. Triggering by the harmonics of the 60 Hz electric power system of North America has been observed more recently (Helliwell, Katsufrakis, Bell and Raghuram, *J. geophys. Res.*, **80**, 4249; 1975). Observations were made simultaneously at Siple in Antarctica and at Robertval, Quebec which is near the aluminium smelting plant at Arvida which uses roughly 1,000 MW at 60 Hz. The rectifiers generate high harmonics, up to about the 90th, that is about 5 kHz, which enter the magnetosphere and trigger emission at other frequencies. The triggering is believed to occur mainly over the equator. The triggered emissions travel to Earth in the whistler mode and can be observed at Siple.

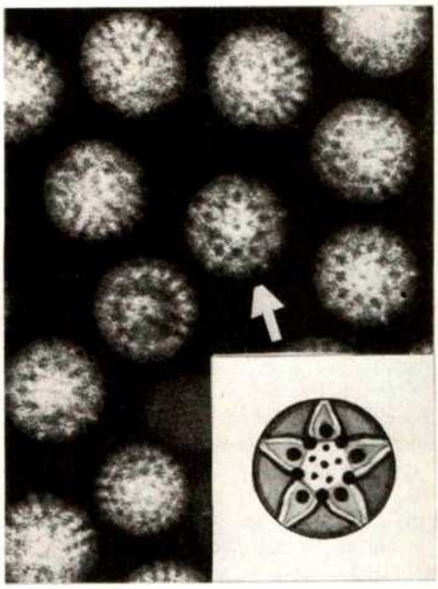
Electromagnetic waves can, in turn, influence the particles of radiation belts so as to change their orbits. One possibility is that the pitch angle of a particle's path is decreased, so that it is made to move more nearly parallel to its guiding line of force. This means that its helical path is more open and it can penetrate further down towards the ground. Then, for many of the particles, mirroring does not occur. They are "precipitated" into the Earth's lower atmosphere, and the radiation belts are correspondingly depleted. Dungey (*Planet. Space Sci.*, **11**, 591; 1963) suggested that electromagnetic signals from the Earth might modify the radiation belts in this way, and there was speculation as to whether the electron slot in the range $2 < L < 3$ could be explained by this mechanism.

A recent study of emissions, mainly in the audio frequency range, made with the satellites Ariel 3 and 4, has greatly strengthened this possibility (see Bullough, Tatnall and Denby, page 401 in this week's issue of *Nature*). The analysis shows that harmonics of the 60 Hz power system in North America might contribute appreciably to the precipitation of particles. VLF radio transmissions from NAA (Maine, 17.8 kHz) and GBR (Rugby, 16 kHz) may also play a part. But thunderstorms in the American and African continents deliver a very large amount of electromagnetic energy into the magnetosphere, and further observations and analysis will be needed before we can be certain that the man-made sources of energy are really significant. ☐

The importance of being rotavirus

from June D. Almeida and Arie J. Zuckerman

LATELY there has been considerable interest in the characterisation by direct electron microscopy of viruses in faeces (see *Nature*, **254**, 557; 1975). Recent publications report reovirus-like agents in stools (Kapikian *et al.*, *Science*, **185**, 1049; 1974), infantile gastroenteritis virus or IGV (Newman *et al.*, *Nature*, **258**, 631; 1975), duovirus (Bishop *et. al.*, *Lancet*, **ii**, 1281; 1973), rotavirus and orbivirus (Flewett *et al.*, *J. clin. Path.*, **27**, 603; 1974). To the uninitiated it might seem that there has been a vast increase in the number of virus types to be identified in acute gastroenteritis. Closer examination reveals, however, that each of these descriptions concerns the same virus, which is now associated with acute diarrhoea in the human infant, the calf, the mouse, the pig and the foal. The outstanding feature of this virus is its morphology and at least three of the terms suggested are based on this morphology. Duovirus refers to the double-shell construction, orbivirus to its overall circular appearance and rotavirus refers to the spoke-like outer projections which give it a distinctive wheel-like appearance. It now seems that under certain circumstances the same virus can reveal a distinctive star-like pattern which is shown in the figure and interpreted in the inset diagram. The structure illustrated was obtained from a specimen of faeces from a calf with diarrhoea. The titre of the particles was particularly high, estimated as being in the order of 10^{11} particles ml^{-1}. Rotaviruses consist of two concentric capsids each built up from distinctive, geometrically arranged subunits. The star-like morphology demonstrated may be a superimposition pattern formed by the interaction of these two structures. On the other hand, it may be that the stellate form shown in the figure is the intact form of this virus, and the ability to distinguish two components implies that breakdown has occurred.



A group of rotavirus particles from a calf faecal homogenate. The majority show the morphology associated with this virus type and both complete and incomplete forms are present. In addition there are two particles showing the star arrangement described. ×225,000. Inset, diagrammatic representation of the stellate particles based on the detail present in a number of them. To be compared with the arrowed particle in the main micrograph.

This finding suggests that the situation could be further confused by the name astrovirus, one already used for gastroenteritis viruses by Madeley and Cosgrove (*Lancet*, **ii**, 451; 1975) or even stellavirus. But rather than introduce yet another name for this already terminologically overladen group it seems better to fit this new morphological finding to an existing term. The diagramatic inset emphasises a certain similarity between the new morphological feature of this virus group and a mediaeval "wheel of fortune". Thus two separate aspects of this virus group exhibit a wheel-like appearance, in turn suggesting that the term rotavirus may well be the most appropriate for this group which seems to move in wide circles throughout the animal kingdom. ☐

Peaks and valleys in morphogenesis

by Miranda Robertson

A symposium on Polarity and Morphogenetic Fields was held at the Hubrecht Laboratory, Utrecht on March 4–6, 1976.

THE main obstacle to progress in understanding polarity and morphogenesis in embryos is the elusiveness of morphogens. One of the latest to confront the problem is Bruce Alberts, working in Lewis Wolpert's laboratory at the Middlesex Hospital Medical School on sabbatical leave from Princeton and DNA replication. He is attempting to isolate the morphogen from the polarising zone of the chick limb bud—an approach which Wolpert, concluding the Symposium, compared to a direct assault on Everest without oxygen. Wolpert belongs to a school which, feeling ill-equipped to scale the heights, has elected to tackle the foothills by constructing physically plausible theories to account for morphogenetic phenomena at the cell and tissue level —and thus elucidate the principles of morphogenesis rather than try to elucidate its molecular mechanism.

The predictive power of one such theoretical construct has been tested by Harold MacWilliams (Albert Einstein College of Medicine, New York), in an experiment aimed at distinguishing between Wolpert's theory of polarity and morphogenesis in *Hydra,* and that of Alfred Gierer and Hans Meinhardt (University of Tübingen).

Wolpert postulates two gradients, one activating and the other inhibitory, both with a source at the head end. The Gierer–Meinhardt model is more complex. It too is based on gradients, giving short-range activation and long-range inhibition of head formation; but it involves a further gradient, in activator source density. In the concrete metaphor used by MacWilliams, source density could be the density of cells synthesising activator, and the short-range activation gradient the concentration of free activator. The pattern of these gradients re-establishes itself after experimental perturbation. But the theory predicts that regulation involving changes in the activation gradient be quicker than those which depend on changes in source density. By means of grafting experiments, MacWilliams has been able to compare the time taken by putative high-source regions and putative high-activator regions to regulate down to the same level of activation. In his experiments, the latter took 12 hours for an adjustment that took the former forty. Wolpert's simpler theory cannot account for this result.

Most of those present at the symposium probably accepted that polarity originates in interacting gradients. Brian Goodwin (University of Sussex) offered an alternative based on work with the large single-celled alga *Acetabularia,* in which polarity seems to reside in the membrane sheath. When the cytoplasm of an isolated section of stalk is removed, homogenised and returned, the cap regenerates with the same polarity as before. Moreover the polarity of the cell corresponds to a gradient of phosphate incorporation on the inner side of the membrane, and exposure to a calcium/magnesium ionophore, which reversibly inhibits differentiation, also abolishes the phosphate gradient. Goodwin would like to attribute the phosphate gradient to a propagated wave of action potentials which can be recorded from an external microelectrode. He has suggested a graded "memory trace" in the form of a graded deposit of material left by a propagating wave to explain the establishment of polarity in multicellular animals. But as he acknowledged, there is no evidence for a causal relationship between the wave and the gradient in *Acetabularia.*

To objections that the origin of polarity in a single-celled organism may be irrelevant to multicellular ones, Klaus Sander (University of Freiburg), chairing the session, responded that all embryos develop from a single egg cell. To explain his own results with insect eggs, however, he has joined the majority in postulating gradients rather than propagating waves.

Sander has shown that the antero-posterior polarity of the chironomid midge *Smittia* can be partly reversed by centrifugation, local ultraviolet irradiation, or even a pinprick at the anterior pole of the egg. This can produce embryos with mirror-image duplication of the abdomen instead of a thorax and head.

This pattern was explained by Meinhardt in terms of the Gierer–Meinhardt theory of short-range activation and long-range inhibition. The effect of disrupting the egg is to allow the inhibitor level to drop, creating a second activation peak. The change in slope of the gradient explains the reversal of polarity. But which sequence of segments is specified depends on the gradient level at the point when it is interpreted by the cell. To explain the exact patterns produced by insect eggs disrupted at different times, Meinhardt has had to add to the theory an account of how the cells read the gradient level. He suggests that genes are sequentially inactivated, the number of genes inactivated increasing with the height of the gradient; but whereas the gradient level can change rapidly on disruption, it is read relatively slowly and because of sequential inactivation, it takes longer to read high values than lower ones. Since in *Smittia,* the gradient is read from posterior to anterior, disruption can thus cause only the specification of more posterior structures.

This particular formulation however works only for size invariant fields. Pattern regulation in insect eggs and *Hydra* is morphallactic: that is, the pattern re-establishes itself by adjustment of existing tissue, without new growth. Willem Ouweneel (University of Utrecht) and Bruce Carlson (University of Michigan) addressed themselves to the rather different problem of epimorphic growth, which occurs in the regeneration of insect and amphibian limbs, and *Drosophila* imaginal disks.

Lively discussion was stimulated by Ouweneel's account of a recent attempt by Vernon French and Susan and Peter Bryant to draw the data from cockroach and newt limbs, and *Drosophila* imaginal disks under the same theoretical umbrella. The theory was developed by French to overcome difficulties in explaining the reliable appearance of supernumerary limbs in the cockroach if one of the transverse axes of the leg is reversed by left-right grafting. Susan Bryant simultanously and independently arrived at the same explanation for analogous effects in newt limbs. The crux of the theory is that the limb is specified by polar coordinates (the "clock-face" model).

Reversal of polarity of the blastema relative to the stump creates a discrepancy between the positional values of blastema and stump at any given location, and the theory states that that discrepancy is eliminated by intercalary regeneration of a series of values completing the normal sequence by the shortest possible route (Fig. 1). Where the discrepancy causes the regeneration of a complete circle, supernumerary limbs will appear. Polar coordinates

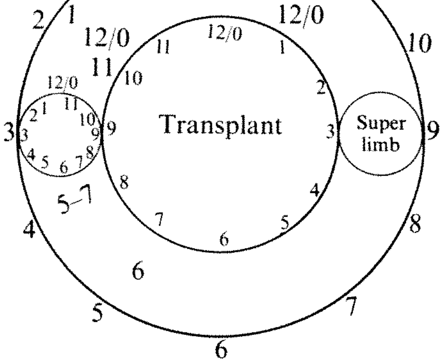


Fig. 1

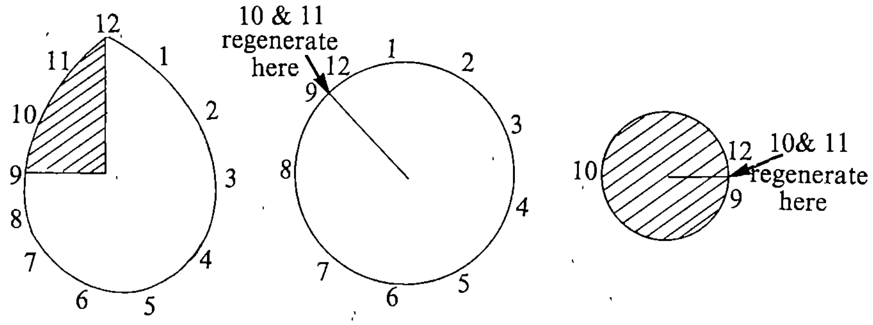


Fig. 2 The wing disk of *Drosophila*. The cut edges of the fragments come together and intercalary regeneration occurs between them.

can also account for the pattern of intercalary regeneration recently reported by Haynie and Bryant for *Drosophila* wing disk (*Nature*, **259**, 659–662; 1976). If a quadrant is removed from the disk and the two fragments allowed to complete their development, the major fragment always regenerates the missing pattern and the minor one always mirror-duplicates the existing one. If the manner in which the cut edges heal is taken into account, intercalary regeneration of polar values accurately predicts this behaviour (Fig. 2). Bruce Carlson's experiments on the regeneration of multiple digits in axolotls give results which however do not entirely conform to the pattern seen in newt blastema. Carlson has explored the effects of rotating separately the different tissues of the stump and finds that the pattern of regeneration is distorted only when mesodermal tissues (muscle or dermis) are rotated. But whereas Bryant finds supernumerary limbs after either antero–posterior or dorso–ventral reversal of the blastema, Carlson obtains multiple digits only from antero-posterior rotation.

S. Strub (Zürich) reported what seems to have been the disruption of the proximo–distal, as opposed to the circumferential, coordinates of the *Drosophila* leg disk by disaggregation of a quadrant of the disk. Such fragments develop entirely distal structures. These are normally specified at the centre of the disk, where French, Bryant and Bryant suggest that the gradient is lowest; but how disaggregation should cause such sinking to a uniform low is not clear.

It was generally agreed that if such puzzles are to be solved by theoretical modelling, the models will have to be formulated in precise quantitative terms, both for the establishment of the morphogenetic gradient and for its interpretation by the cells. Richard Gardner (University of Oxford) pointed out that whereas for example the homoeotic mutants of *Drosophila* could be used to shed some light on the cellular response, mutants affecting the establishment of gradients are unknown. The best that models can do is to provide logically consistent quantitative descriptions of experimental phenomena, as long as the molecular mechanisms of morphogenesis remain inaccessible. It is reported that a British Forces team will shortly attempt Everest without oxygen. □

Inverse square law for gravity?

from a Correspondent

'EVERY schoolboy knows' that gravitational attraction varies inversely as the square of the distance between the attracting masses—or at least it used to be common knowledge. Newton himself first gave strong support to the 'inverse square law' by comparing the rate at which the Moon accelerates towards the Earth with gravitational acceleration at the Earth's surface. He later provided compelling evidence by showing that Kepler's laws for the motion of the planets followed from the same law of attraction—that is, that they would move on ellipses with the Sun at one focus, and the square of their periods would be proportional to the cube of their major axes. Subsequent study of the motion of the planets and satellites has confirmed the truth of the inverse square law at astronomical distances (say from 1,000 km upwards) to a high degree of precision. It is therefore intriguing that in an article by D. R. Long in this issue of *Nature* (page 417), the question of the validity of the inverse square law at laboratory distances has been raised. An examination of the most accurate laboratory determinations of the constant of gravitation, G, suggests a systematic shift with the separation of the attracting masses—the larger the separation, the larger the value of G. In other words, Long suggests that the inverse square law is not valid, and that we have to add an additional short-range repulsion. The experiment reported here confirms his suggestion, by comparisons at distances of 4.5 and 30 cm.

The question of a deviation from the inverse square law has been raised most seriously in 'recent' times by Newcomb, who showed that a small change in the form of the law could explain anomalies in the motion of Mercury. These anomalies are explained naturally by Einstein's General Theory of Relativity, which may be interpreted in a sense as predicting deviations from the inverse square law of gravitation. However, at laboratory distances with laboratory masses, Einstein's theory predicts that the inverse square law should hold to a high precision. Long's suggestion will therefore not meet with ready acceptance. If substantiated, the implications, for example, for our knowledge of the interiors of the Earth and the Sun would be considerable. We infer their masses from our knowledge of the constant of gravitation, and Long's experiment indicates that the limiting values of G at large distances may be several percent higher than is presently accepted. Moreover the implications for a quantum theory of gravity would be serious.

One is tempted to echo the reaction of the bishop's wife to the suggestion that man is derived from the apes—'let us hope that it is not true—or if it is, that it won't become generally known.' More responsibly, we should affirm our willingness to submit theory to experimental proof, and hope that Long's suggestion will stimulate a critical experimental appraisal. He has drawn attention to the remarkable fact that there is no accurate experimental evidence for the inverse square law of gravitation at laboratory distances. By comparison, very accurate null experiments on the basis first indicated by Cavendish have shown that the exponent in the inverse square law for electrostatics differs from two by less than 3×10^{-16} (Williams, Faller, and Hill, *Phys. Rev. Lett.*, **26**, 721; 1971). Can anyone devise an accurate null experiment which will confirm (or deny) the inverse square law for gravitation ? □

Erratum

In the article 'Disappearing habitats' (*Nature*, **259**, 365; 1975) the floral survey mentioned was carried out by Mr and Mrs C. Scotter and not by P. M Wade.

Mason develops Milankovich Ice Age theory

from John Gribbin

ON March 17 Professor B. J. Mason, Director-General of the UK Meteorological Office, delivered this year's Symons Memorial Lecture, in which he presented strong evidence in favour of the 'astronomical' theory of Ice Ages, with his own improvements on the Milankovich model.

Mason's talk, titled "Towards the understanding and prediction of climatic change" covered some familiar ground both at the beginning, where he restated his position in the debate about whether the sunspot cycle and other solar fluctuations affect climate (see *Nature*, **259**, 367; 1976) and at the end, where he stressed the value of computer modelling in our developing understanding of the atmosphere. Professor Mason believes that this is the only way in which we may be able to understand how the climate works although there is a long way to go yet before unique predictions will be possible. In some ways, the centrepiece of the talk was also familiar, at least to readers of *Nature*, in that it dealt with the Milankovich theory of Ice Ages. But Mason presented new, unpublished evidence in support of the theory, and also offered a uniquely persuasive insight into how the energy fluctuations involved could drive both the onset and decline of Ice Ages; it may well be that as a result, March 17, 1976 is remembered as the day when Milankovich's model, after four decades of controversy, at last became established as the leading contender to explain climatic fluctuations with timescales from a few tens to a few hundreds of thousands of years.

The key to this development has been the availability in the past few years, for the first time, of reliable continuous records of both temperature and total ice volume fluctuations covering the past 10^5 yr or more. These are the records obtained by interpreting isotope fluctuations (notably the ^{18}O/^{16}O ratio) in both ice cores and in marine cores containing the remains of small creatures such as plankton. The three separate variations in Northern Hemisphere insolation produced by changes in the orbit and inclination of the Earth have periods (in round terms) of 96,000 yr 40,000 yr and 20,000 yr; all three of these periods (and no others as significant) were present in a power spectrum analysis of the planktonic data which Mason had obtained from N. Shackleton in Cambridge (as yet unpublished). Mason stressed the importance of this link between statistically significant periodic variations and a known physical mechanism, contrasting the situation with that of some of the more short term data where seemingly significant peaks often have no physical explanation, and peaks expected on physical grounds (such as the 11-yr solar cycle influence) often are not significant. With this incentive, Mason has reworked some aspects of the Milankovich model in terms of variations in the amount of heat received at different Northern Hemisphere latitudes in different seasons, using both the original astronomical data of Milankovich and modern improvements on the data. North of 45°N the variations due to these effects cover about 4×10^{18} calorie d^{-1} (1.5×10^{21} calorie yr^{-1}), which is about 1% of the total heat received by the polar cap, and Mason reported that he was "staggered" to find the very exact agreement over the past 150,000 yr between times of minimum insolation and the maximum advance of Northern Hemisphere ice.

Mason says "this is impressive, and the last thing I expected" and that the remarkable agreement encouraged him to look at the integrated effect of insolation variations during periods in which the ice cover advanced and retreated. Again for the region north of 45°N, when ice cover developed during the period from 83,000 BP to 18,000 BP the integrated deficiency in insolation produced by Milankovich variations amounted to some 1,000 calorie for each gram of ice formed using Milankovich's figures, or some 556 calorie g^{-1} with the modern figures. Since the latent heat of the water/ice transition is 677 calorie g^{-1}, the figures are impressive even without allowing for the possibly crucial significance of the fact that the variations in insolation are much greater in summer than in winter.

From 18,000 to 6,000 BP, when the ice was melting, the integrated 'excess' heat received was 4.2×10^{24} calorie on Milankovich's figures and 10×10^{24} with the modern astronomical figures, while the latent heat required for the known decrease in volume of the ice was 3.2×10^{24}; a coincidence which stretches across 24 orders of magnitude to agree so closely is certainly not to be dismissed lightly, and work is now in progress at the Meteorological Office to produce a more detailed model taking account of the capacity of the ocean to act as a reservoir of heat and the feedback mechanisms between ice and ocean. Mason even ventured if not a prediction then at least a comment on future climatic fluctuations, pointing out that we are now shifting from a situation in which the Milankovich effect has produced excess insolation in the North (above the mean for the past 150,000 yr or so) to one in which there is a deficit. Simple astronomical calculations show the dip reaching a Northern Hemisphere insolation minimum in about 10,000 yr, but bottoming out before reaching conditions quite so extreme as those which coincided with the maximum advance of the most recent Ice Age. Many members of the packed audience for Mason's lecture will undoubtedly be eagerly awaiting the full model now being developed by A. Gilchrist and others at the Met. Office; some, no doubt, will now be taking a fresh look at the Milankovich model themselves. Meanwhile, in Mason's words, "this effect cannot be laughed away... certainly all the energy quantities are in the right ball park". □

A hundred years ago

LORD SALISBURY, on Monday, named the following as Commissioners under the Oxford University Bill: —Lord Selborne (Chairman), Lord Redesdale, the Dean of Chichester, Mr. Mountague Bernard, Sir Henry Maine, Mr Matthew White Ridley, and Mr. Justice Grove. The feeling among scientific men is one of intense disappointment, leading to the conclusion that it is useless any longer to consider whether Oxford will ever be in a position to do anything for the promotion of science.

THE report of the Cambridge Board of Mathematical Studies to the Studies' Syndicate contrasts with the reports of most of the other boards in the paucity of its suggestions for improvement. They do not seem to think that very much is required in order to perfect the system of mathematical teaching. They believe in the probable stability and development of the system of intercollegiate lectures, but say very little to assist its development, and they say nothing about the present vehement competition by means of private tuition, and the defective method of study that it induces. In answer to the question how university teaching may be organised so as to give the greatest encouragement to the advancement of knowledge, "the Board offer no suggestions under this head." Is this quite what might have been expected in a report bearing the signatures of Stokes, Cayley, Adams, Clerk-Maxwell, Sir W. Thomson, Tait, Lord Rayleigh and James Stuart? May there not be some unobvious explanation of this phenomenon? The whole report consists of only forty-one lines.

from *Nature*, **13**, March 30, 434–435; 1876 •

articles

Visual illusions in geology

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Examples of visual illusions in the laboratory are well documented, but what about illusions that occur during field observation of geological structures? In this article I discuss a study involving geologists and non-geologists and define some of the problems that may arise during geological field and laboratory work.

THOUGH visual illusions have been studied mostly in controlled laboratory situations[1,2], Poggendorff, Zöllner and other illusional distortions can also occur during observations of rock formations. Depending on the interaction of psychological and geological hypotheses, these can lead to false deductions. Problems may arise especially in the observation of elliptical ooids, crenulation cleavages and intersecting veins and fractures. Illusions in geological contexts are of psychological interest: judgements of area, for example, are found to be the reverse of those predicted on the basis of the Helmholtz irradiation illusion.

Four versions of the Poggendorff illusion in geological contexts are shown in Fig. 1. The inclined traces in Fig. 1*b* seem to be offset across the stippled bands. Were the bands representative of veins then these illusory offsets could lead to an interpretation suggesting dilational origin, whereas replacement is more appropriate. To some observers the linear elements seem also to be slightly rotated anticlockwise in the centre gap. This could be taken to imply the operation of shear strain of a different magnitude within and outside of the stippled layers. In Fig. 1*c* the rock cleavage traces seem to be displaced by interfacial shear at the layer boundaries, especially at the arrowed locations. The illusory displacements in Fig. 1*d* occur across the dark layers, suggesting a greater independence of the strain histories of the lighter layers above and below, than is justified; in fact, the cleavage traces are precisely in line.

The context effect of the fault at *A* in Fig. 1*a* and the gap in data caused by, say, a river course, increases the probability of an incorrect fault inference at *B*. The stippled layer is straight and continuous. For purposes of terminology in psychological studies it is convenient, however, to take the data gap band in the Poggendorff illusion as analogous to a geological fault. Then the displacement directions in this illusion can be described by labels which are usually reserved for tear faults. The line on the far side of the data gap is sinistrally displaced ('sinistral Poggendorff') when the apparent offset is to the left, dextral when it is to the right. Although such illusory offsets and rotations can be minimised by viewing along the interrupted linear elements—with the direction of view positioned at a low angle to the rock surface—this is rarely done when observing cleavages, since refraction is expected.

When exploratory mineral and petroleum investigations are at the reconnaissance stage, the use of image analysing computers for percentage area estimation is uneconomical. Visual estimations of rock porosity and percentage volume occupied by a diagnostic mineral are frequent, and monochromatic[3,4] and chromatic percentage area comparison charts have been devised to facilitate judgment. But monochromatic charts invariably present black grains on a white background, and single black shapes on white appear smaller than equal area white shapes on black (Fig. 2).

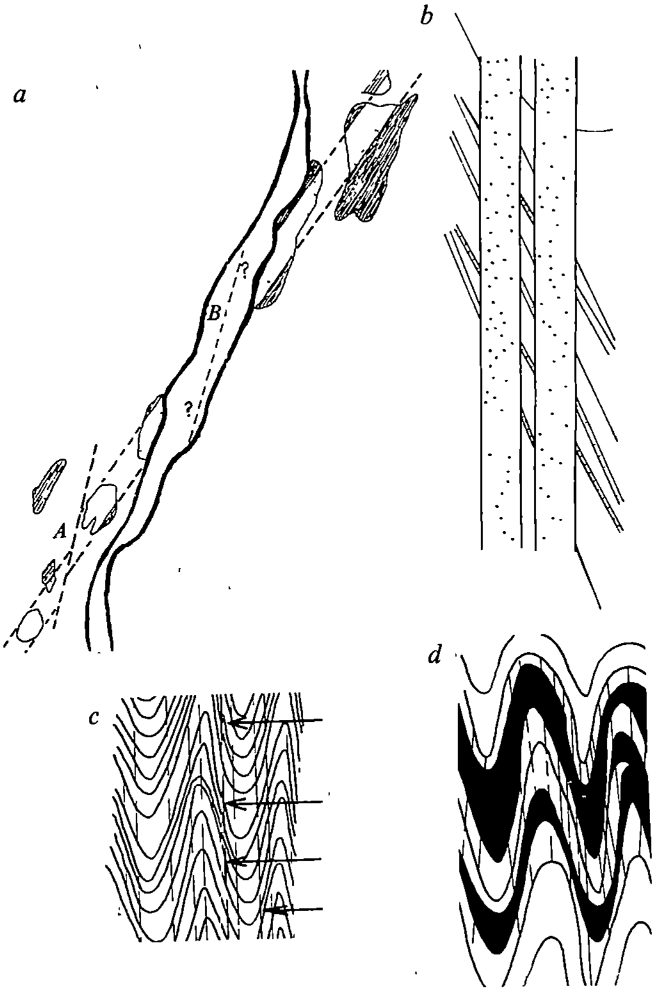


Fig. 1 The Poggendorff illusion in geological contexts; the illusory perceptual data are more likely to mislead when they favour an investigator's geological hypothesis than when they do not. *a*, 'Sinistral Poggendorff' offset of stippled layer across data gap misinterpreted as a fault, an interpretation reasonable at *A*, but not at *B*; *b*, illusory displacements, continuations and rotations of linear elements across two adjacent data gaps suggest dilation effects; *c*, illusory step-like displacements of cleavage, especially at locations arrowed; *d*, apparent offsets of continuous cleavage traces across dark bands.

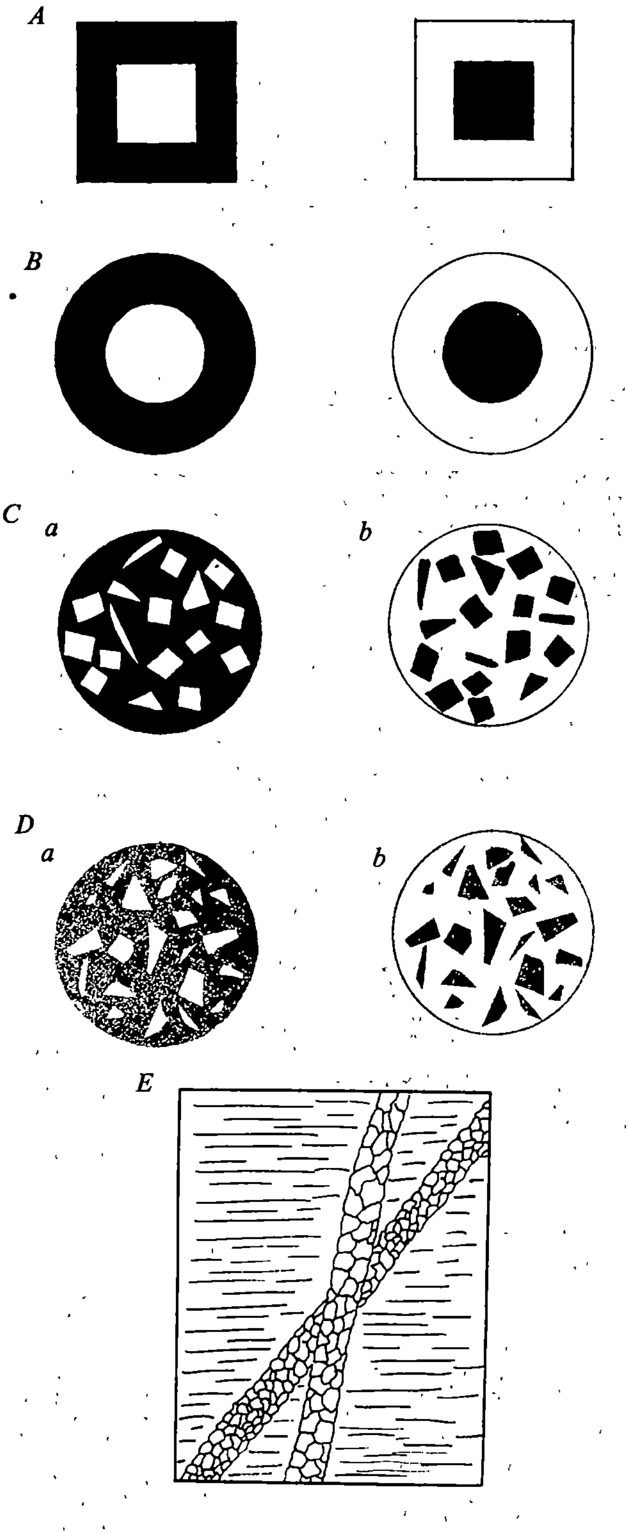


Fig. 2 *A* and *B*, The Helmholtz irradiation illusion in which a light area on a darker background appears larger than an equal dark area on white; however, in *C*, estimates of the percentage area occupied by grains in *a* and *b* are usually equal when both are presented together; but when presented separately the black grains (on the line-bounded background) are judged to cover a larger area than the white grains (on a contrast-bounded background); *D*, the grains in *a* and *b* again, in spite of the lower brightness contrast than in *C*, seem to cover the same area, but the dark grains are judged to cover a greater area when judgments are made separately. Actual area in *c*: 25%; *d*, 20.5%. *E*, 'Sinistral' Poggendorff offset of the older vein in *E* was misremembered as dextral by 23% of geologists.

Although this may be expected to cause an overestimation bias in area estimations of lighter grains, or areas, within a darker matrix, this is not necessarily the case. Twenty-eight geologists (with 2–5 yr experience) individually gave unaided absolute estimates of the total areas occupied by grains in eight 5-cm diameter items (9–23 grains in each). Two of the samples (17 and 23 grains) were presented deliberately both in white on black and black on white, with the grains evenly distributed. Contrary to predictions from the effect in the Helmholtz irradiation illusion, mean area estimates were larger (and not smaller) for black grains on a white background than for white on black. Overall mean values were respectively 29.4% and 25.4% ($0.025 > P > 0.01$, actual area 25%). Over this range of grain density the act of estimation may therefore involve other perceptual and cognitive processes which cause closure between multiple black elements and which thus counter the mechanisms used in seeing the standard irradiation illusion.

Monochromatic charts with black-on-white figure–background relationships should be misleading when total area estimations need to be made of a few large items; they do not accord so closely with estimates made without their aid as do the white-on-black scenes. The latter, in fact, are less likely to cause an underestimation bias for scenes of intermediate magnitude. Imagery-based techniques, such as mentally translating grains so that they cluster and fuse into a sector, do not promote greater accuracy of performance. Subjects stated that mental translation led to increased errors when the grains were small, widely separated, circular, or of uneven outline. Estimation through general impression, using 3–4-s delayed augmented feedback during 15 estimations of chromatic and monochromatic natural and diagrammatic samples, led to lower errors in other subjects after unaided estimates had stabilised (before–after comparison for 15 geologists (> 3 yr experience) and 2 non-geologists (one male, one female), ($0.025 > P > 0.01$)). The two female subjects tested had the highest initial errors but improved more than all the male subjects, eventually equalling male performance.

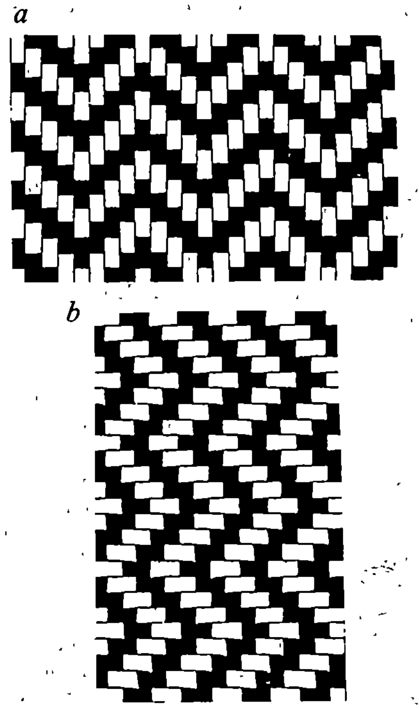


Fig. 3 *a*, The "illusion of the kindergarten patterns" is schematically similar to fracture cleavage associated with large offsets. The apparent fanning of the laths is entirely illusory; all are parallel-sided and aligned vertically. Decreases in brightness contrast between, say, lighter sandy layers and darker clay-rich layers, decreases the illusion. *b*, The same as *a* but at 90° to it.

If illusory data elements contradict common geological expectations, then the latter dominate over some of the psychological elements, during constructions in visual memory, but not necessarily over others. A total of 69 geologists with 2–5 yr experience observed a slide depicting two acutely intersecting but straight, undisplaced veins of equal thickness (Fig. 2*e*). The intended Poggendorff offset is sinistral. After viewing for 7 s they drew representations which were assessed by three neutral judges for unambiguity of age relationships and true continuity of the older (more gently inclined) vein across the younger (near vertical). Twenty subjects did unambiguously represent the older vein as displaced but 16 of them showed the displacement as dextral. Such a representation should be expected only if the younger vein grows at a high angle to its margin. Six geologically and psychologically inexperienced subjects reported seeing the "sinistral displacement" but four of these could not accurately remember data on age relationships (the latter was accurately recorded by 96% of the geologists).

Figure 3 shows the combined effect of the irradiation distortion and Poggendorff offset in the form of the "illusion of the kindergarten patterns"[5]. This is schematically similar to those instances of fracture cleavage in deformed rocks where the displacement along the cleavage is large in comparison to the thickness of the cleaved layer, and the brightness contrast of the rock lithologies is high. The 'fanning' of the cleavage is illusory: all the cleavage surfaces are straight, parallel and vertically in line. Similar problems may be created by geological analogues of the Zöllner and Fraser illusions (ref. 2 and Fig. 4). The tightly folded recumbent multilayer in Fig. 4*a*, for instance, seems to be penetrated by a convergent cleavage fan; that in Fig. 4*b* seems to be penetrated by divergent zones of thinning. In fact, the cleavage traces are parallel and in line (that is, 'axial planar'). Structures with these general appearances are quite common in orogenic belts, yet true axial planar cleavage is rarely reported. Its frequency of occurrence may have been underestimated. This emphasises the importance of the precise measurement of cleavage fan angles, in contrast to reliance on visual judgment and general verbal labels.

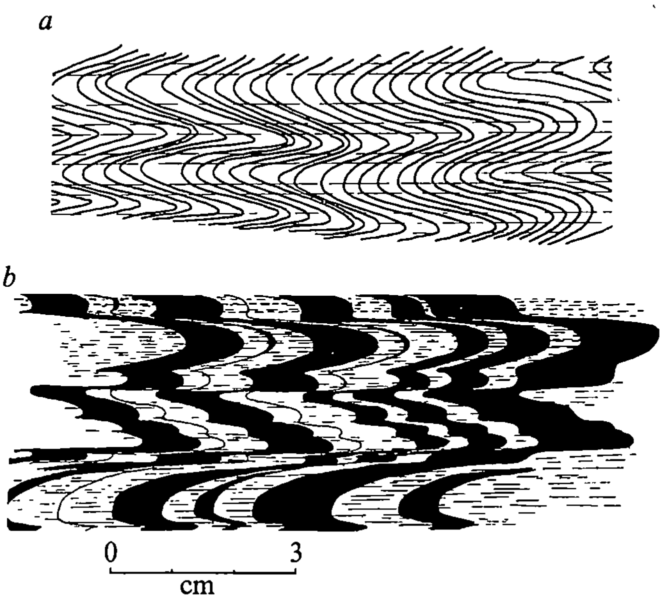


Fig. 4 Geological analogues of (*a*) the Zöllner illusion, and (*b*) the Fraser illusion. *a*, Recumbent multilayer folds seems to have an associated convergent cleavage. In fact, all the cleavage traces are parallel (true axial planar cleavage) Easily overestimated convergence angles are also seen in many outcrops of mesoscopic F_2 folds in the area of the Ballachulish "nappe", Argyllshire, Scotland. *b*, Based on a natural structure in Appin Quartzite Transition Group, Laroch Valley, Argyllshire. Six out of seven postgraduate structural geologists perceived the four major crenulation cleavage zones as fanning, and the finer, small scale cleavage traces as parallel sided. In fact, finer traces are fanning (divergently) and lower order zones are parallel sided.

In the vertical–horizontal illusion a vertical or slightly left-of-vertical line[6] appears longer than a horizontal line of the same length. Consequently, the amplitude–wavelength ratio of standstone ripples, or indeed any waveform, seems to be larger when the enveloping surface to the waves is horizontal, than when it is vertical. Low eccentricity ellipses, with horizontal long axes also appear closely circular when the difference between axes lengths is small (< 5%). Geological objects known as 'ooids' (Fig. 5*a*), which have circular outlines when undeformed, become elliptical after compaction and tectonic deformation. They are, however, rarely geometrically ideal shapes, and in some cross sections their interiors may be very irregular or even more elliptical than the peripheries. In the latter case this results in an illusory contraction of the ooid normal to the long axis of the interior ellipse—a variant of the Delboeuf illusion[7].

In most real geological situations, however, specific and nonspecific context effects do influence perceptual judgments. An important nonspecific effect is the continual necessity for separating out non-random fluctuations in shape and orientation from the inevitable random visual 'noise' inherent in geological (as opposed to geometrically ideal) shapes. Signal non-randomness in ooid geometry can go undetected when axes lengths differ by between 5% and 15% if geologists, through training, become accustomed to perceiving non-ideal forms and thus too easily accept natural shape irregularities as noise. Out of 54 professional and postgraduate structural geologists asked individually if the ooids in Fig. 5*a* needed analysing for evidence of deviations from circularity and of non-randomness in orientation, 36 replied that they did not (Fig. 5*c*). In fact, all the ooids are elliptical (Fig. 5*b*) and there is a preferred orientation of long axes in the horizontal. This fabric would not be eliminated no matter how high any subsequent strain and so their analysis is necessary. Sixteen non-geologists asked the same question all replied that they did think the ooids required analysis (confidence levels $\geqslant 3$). This would be explicable if the non-geologists were comparing the stimuli with more ideally shaped, memorised prototypes.

The practical way to aid decisions in these circumstances is for the observer to experiment with himself by judging the axial ratios of those ooids which to him seem closest to a unit axial ratio (however irregular their peripheries) and then checking the judgment by measurement. Errors of up to 15° commonly also occur in the location of the long axes of ooids when axial ratios are below 1.3:1. If two possible 'long axes' are constructed at the limits of the zone of location uncertainty, the length of the bisector of these axes is a better measure than that of one located intuitively.

On the basis of inappropriate constancy scaling interpretations of illusions[1], the upper half ('further away') portion of a vertical line should appear longer than the lower half. In addition, subjects can be expected to locate mid-points too high up along a vertically oriented dimension. Some evidence for the presence of such an effect, in a geological context, arose during an investigation[8] in which professional geologists located inflection points too high up on the limbs of moderate and high limb dip folds. Bisections were made within zones ranging from < 0.5 cm to 3 cm in length. In order to test both for range effects and also for possible psychological hindrances in the use of an additional way-up determination technique (Fig. 6), 30 final-year undergraduate geologists were requested to locate a number of mid-points between parallel lines separated by 3 cm and 6 cm. They were to judge the mid-height point. The parallel lines could be horizontal, inclined (45°) to the left, inclined to the right or vertical. Each of the 30 subjects provided 2–10 observations for each of the 8 conditions. No bias was found for the oblique or vertical cases, but in both width conditions, there was a small but very consistent tendency to locate mid-points too high up between horizontal lines (mean error 1.6%, $0.01 > P > 0.001$; all other conditions showed mean non-constant location errors of < 1%).

In an experiment examining the detection of asymmetry

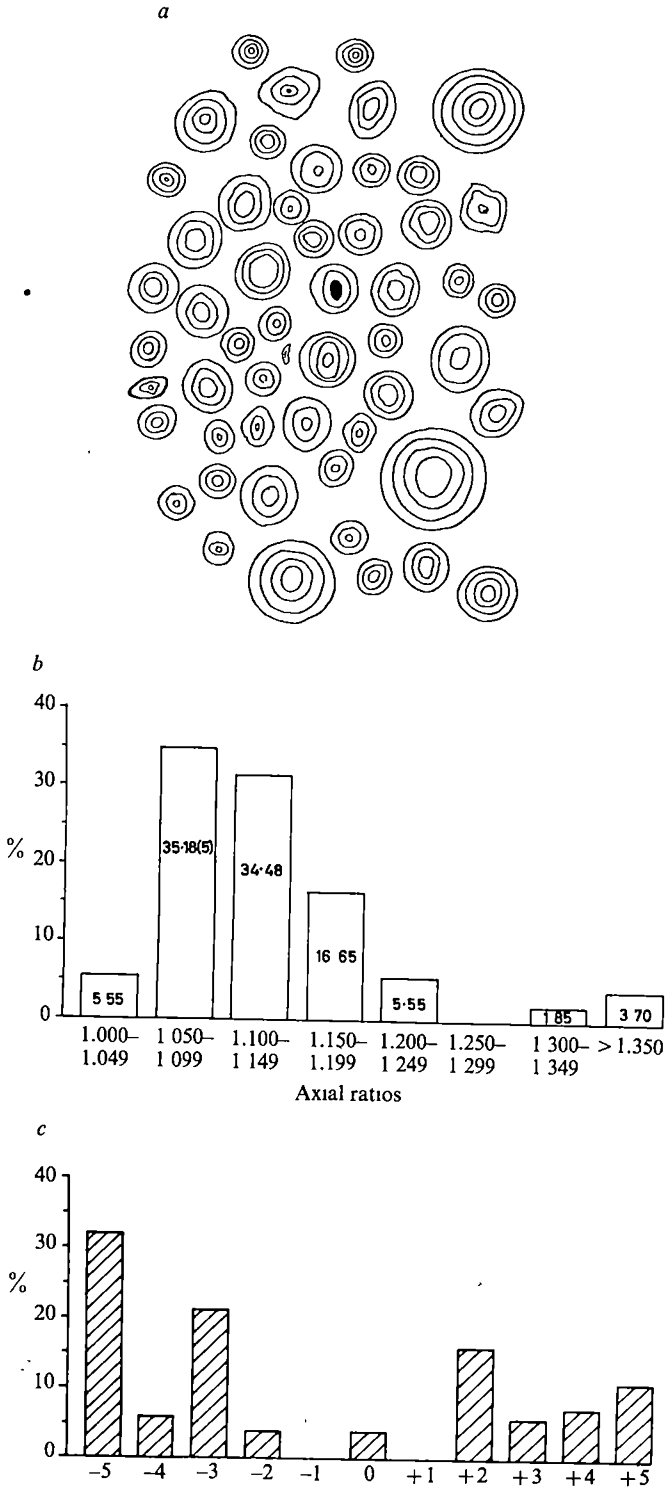


Fig. 5 *a*, In reflection of the vertical–horizontal and Delboeuf illusions, slightly elliptical ooids appear more closely circular than really they are. *b*, Frequency histogram of ooids' axial ratios, none of the ooids in *a*, are circular (54 ooids, mean axial ratio 1 15, range 1.025–3.45). *c*, Decisions of 54 geologists as to whether or not to analyse the ooids in *a* for evidence of inherited non-random deviation from a circular outline (−5 to +5, increasing levels in confidence of decision: −5, "definitely would not analyse"; +5, "definitely would analyse").

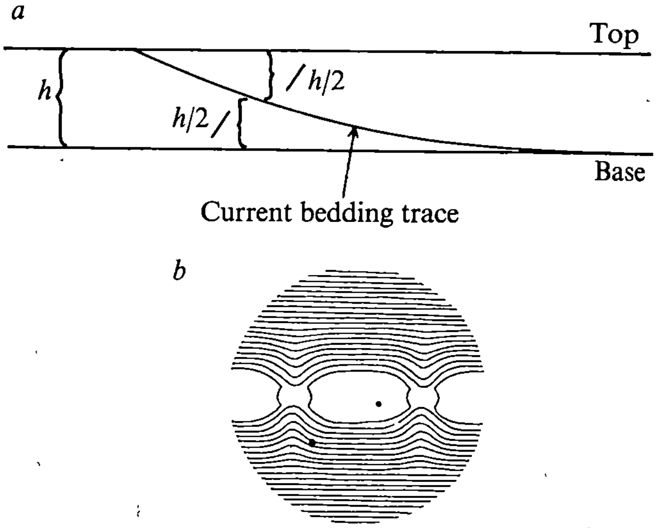
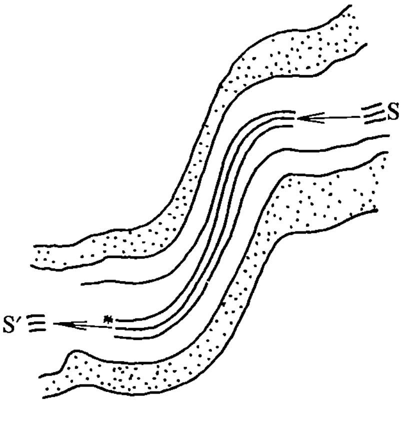


Fig. 6 *a*, 'Mid-point' method of way-up determination in areas where younging evidence is sparse and current bedding strongly flattened, demonstrated on an unambiguous example. Where mid-height surface intercepts current bedding trace, the longer segment is in the older half of the bed. Locations of mid-points by eye in horizontally positioned units tend to be too high up, leading to incorrect inferences of younging direction. *b*, One of the boudinaged layer stimuli: if deflections have less penetration above a boudinaged layer than beneath, this asymmetry is less likely to be detected than if the converse is true

in boudinaged layers (Fig. 6*b*), similar results were obtained when 10 non-geologists and 3 postgraduate geologists assessed whether the zones of deflection around boudinaged layers (the waves dying out on both sides of the broken layer in Fig. 6*b*) had the same or different distances of penetration (stimulus visual angle 12°). Each subject provided two observations for each of four width conditions (same, 10, 20 and 50% difference) and four orientation conditions (horizontal, left and right oblique, vertical) in tachistoscopic presentation. The mean accurate decision time for the horizontal cases was 4.8 s, but for both oblique and vertical cases was 4.6 s. When, as in Fig. 6*b*, the upper zone of the horizontal condition was 10% narrower than the lower, 31% of the responses were erroneous; when the two were equal, 27% were erroneous. But when the lower half was the narrower, errors were reduced to 11.5%. This also indicates that when waves with progressive amplitude changes, cross the vertical dimensions to be compared, misjudgments of the length differences become exaggerated. The processes causing this may include differences in the implicit use of cues to upward as compared with downward perspective changes.

Provided that these perceptual difficulties are considered, it is possible for the geologist to use the mid-point location as an aid to way-up determinations. When current beds are strongly flattened it becomes more difficult to differentiate between their asymptotic bases and truncated tops. The way-up can, however, be determined by locating the mid-point between

Fig. 7 During lengthy visual tracking of layers in air-photographs and outcrops eye movements occasionally "jump tracks" and continue along adjacent layers. During visual tracing of the layer *S*, starting at top right, eye movements may continue at bottom left along *S′*. Correct line is asterisked.



the flanking bedding traces. Where this mid-height surface intersects the current bedding trace it divides it into two unequal segments, and the longer segment is in the older half of the bed (Fig. 6). I have validated this technique on flattened current beds in the Moine and Dalradian quartzites of the Loch Dochard region of Argyllshire, Scotland. If, however, the flanking bedding traces are horizontal then the mid-point between them should be located by measurement.

If illusions are conceived in terms of inappropriate visual induction processes, then they are difficult to compensate for because the false hypotheses on which they are perceived are in other circumstances correct, useful, and also out of conscious control[1]. Hypotheses, some of which can be brought into awareness, are also generated during visual search[9] and visual pursuit. In Fig. 7 a common assumption is that the three-layer batch at top right continues at bottom left. On the basis of this incorrect hypothesis the observer's eye movements shift from S to S'. When a wave of this geometry was present in a longer pursuit task, it caused shifts in eye movements, to adjacent layers, in 9 out of 29 undergraduate geologists. Visual pursuit needs to be done accurately during the interpretation of air-photographs and also during the observation of complex refolding relationships in outcrop. In the latter circumstances, pursuit lapses can lead, for instance, to 'mushroom' interference patterns[10] (contorted, but closed outcrop patterns) being missed when present, or spuriously inferred when absent. Because of difficulties in the visual pursuit of schistosity through the zone of thinning, crenulation cleavage often seems to involve actual limb displacements in items where no such displacements exist. Observers' eye movements (saccades) shift from their intended course, especially when the track is hypothesised to be more predictable than really it is. To avoid these shifts it is useful either to look along the track at a low viewing angle or deliberately to concentrate on the minutiae of any irregularities. The latter technique reduces saccade distance through eliminating the hypothesis that the track is highly predictable.

Received October 29, 1975, accepted February 18, 1976.

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Man-made e.l.f./v.l.f. emissions and the radiation belts

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Morphological studies of e.l.f./v.l.f. emissions on the Ariel III and IV satellites indicate that man-made electromagnetic emissions, namely power-line harmonics generated in the industrialised regions of North America and, also, v.l.f. transmissions at 17.8 kHz (NAA) and 16.0 kHz (GBR) in the longitude sector which encompasses the South Atlantic Anomaly, are responsible, at least in part, for the formation of the electron slot ($2<L<3$) between the inner and outer radiation belts in the magnetosphere.

A PRELIMINARY study by Lefeuvre and Bullough[1] of v.l.f. emissions at 3.2 ± 0.5 kHz and 9.6 ± 0.5 kHz recorded on the Ariel III satellite (altitude $\sim$500 km, orbital inclination 80°) revealed the existence of a permanent zone of v.l.f. emission occurring over the USA and its geomagnetically conjugate region in the Southern Hemisphere. These Ariel III studies have since been extended and now confirm the importance of this longitude sector for wave-particle phenomena at low L shells ($2<L<3$). This American zone of e.l.f./v.l.f. emission is shown most clearly on the maps of percentage occurrence (Figs 1 and 2) for quiet magnetic periods ($K_p \leq 2+$). To obtain Figs 1 and 2, a count was made of the number of observations (typically 25–40) in each $10^{\circ}\times 2^{\circ}$ longitude–latitude sector and the percentage of such occasions for which the signal intensity exceeded $10^{-11}\ \gamma^2\ \mathrm{Hz}^{-1}$ FSE found. (FSE: free space equivalent, thus $10^{-11}\ \gamma^2\ \mathrm{Hz}^{-1}\ \mathrm{FSE} \equiv 4.8\times10^{-15}\ \mathrm{W\ m^{-2}\ Hz^{-1}}$ for a circularly polarised wave.) The orbit of Ariel III was such that full local time coverage was obtained in each 80-d period analysed. Little importance can be attached to the very localised maximum at 5°E, 56°N which occurred in the quiet period following a magnetic storm and close to a ground station at Winkfield, UK where observations were limited by tape recorder read out.

In Fig. 1 the 20% contour of occurrence is centred rather accurately on the North American continent (shown dashed) with a corresponding conjugate region in the south. The 60% contour in the Northern Hemisphere mapped geomagnetically into the opposite hemisphere shows isomorphism in the two conjugate regions. There is a pronounced maximum in occurrence (>60%) on lower L-shells ($2<L<3$). A closer examination of the contours of occurrence over North America (Fig. 2) shows that it is possible to attribute the source of the emissions to a combination of power-line harmonics and thunderstorm noise. The two major centres of thunderstorm occurrence (Colorado Mountains and the south-eastern USA) are four times more active in the first period (1967 May, June, July) than in the second (August, September, October). In the latter period the 60% contour is situated above the most highly industrialised region of the USA and south-eastern Canada. In the earlier period its extension to the west and south could be attributed to the greater thunderstorm activity. Electrical power consumption in Canada is concentrated in the south of Ontario and Quebec. Available statistics do not enable us to display it on the map. Measurements by D. Morgan (private communication) in a comparable industrialised area in the UK yielded magnetic field strengths at the ground of about $10^{-11}\ \gamma^2\ \mathrm{Hz}^{-1}$ for the 3.2 ± 0.5 kHz channel. This is the same as the baseline we have used for Figs 1 and 2. For typical high thunderstorm activity the mean thunderstorm noise level, measured overhead on the Ariel IV satellite without wave amplification, is also $\sim 10^{-11}\ \gamma^2\ \mathrm{Hz}^{-1}$ FSE. A preliminary examination of 500–1,000

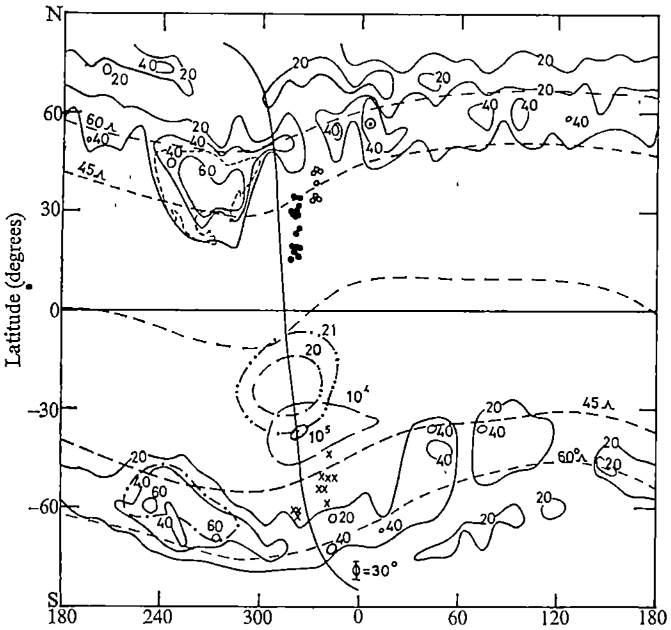


Fig. 1 Percentage occurrence of emissions with intensity $>4.8\times10^{-15}$ W m^{-2} Hz^{-1} at 3.2 kHz; 1967 May 5–July 24, $K_p \leq 2+$. — • — •, 60% contour (Northern Hemisphere) mapped into Southern Hemisphere (magnetic conjugate). – • • – • • –, Brazilian magnetic anomaly [magnetic field at 600 km ($\times10^{-6}$ T)]. – – –, South Atlantic anomaly [omni-directional electron flux >0.5 MeV cm^{-2} s^{-1} at 200 km (Vette, 1967)]. ●, 1967 May 16–23; ○, 1967 June 7–8 inclusive [GBR (code)]; ×, 1971 December 12–1972 March 1 [NAA (FSK)].

Hz e.l.f. data from Ariel IV (1972 March 20–June 22) indicates a small maximum in occurrence for signals $>10^{-11}$ γ^2 Hz^{-1} FSE (but none for signals greater than 10^{-8} γ^2 Hz^{-1} FSE) of 85–90% compared with an average of 65% around the world (at $L = 3$). Amplification at e.l.f. is much less than at v.l.f. (see below) and the signal could correspond to unamplified power-line harmonics. Thus the amplified emissions may be band limited in agreement with the rather limited observations from OVl-14 (ref. 2).

Dependence on geomagnetic activity

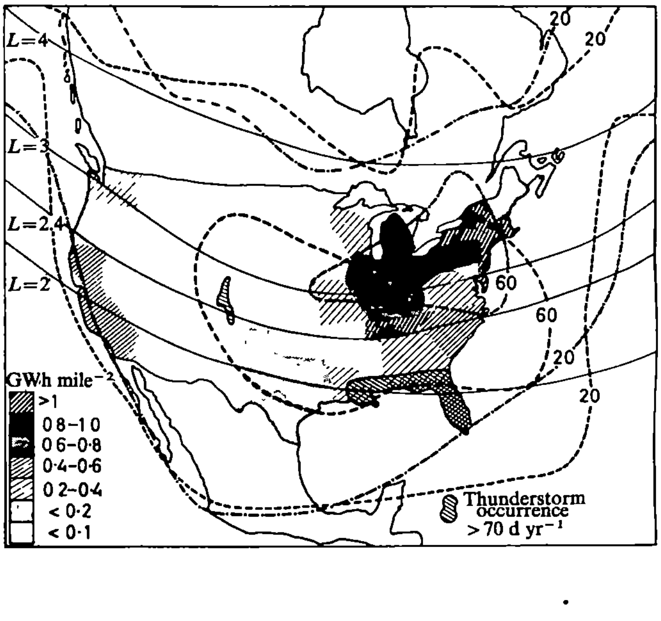
A detailed analysis of all passes over the zone maximum (240–300°E, 30–45°N) for both quiet and disturbed periods revealed a wide range in signal intensity (~50 dB) which reflects the variation in magnetic disturbance ($K_p = 0+$ to 9o) in this period. All measured intensities lay above the receiver threshold and a 10–20-dB reduction in the weakest signals from night to day could be ascribed to D-region absorption of a signal transmitted from the ground; this signal level being that obtained in the absence of amplification during quiet periods. Because of this wide range in signal intensity it was not possible to identify either a diurnal or weekly variation in signal intensity which could be attributed to the expected variations (≤ 3 dB) in power consumption. It is clear that wave amplification (two-hop whistler signal for a source in the USA) occurs very frequently. We have not yet analysed the one-hop whistler signal for this period. An analysis similar to that of Fig. 1 but for disturbed periods ($K_p \geq 3$o), shows the percentage occurrence rising to 100% over the eastern USA and its conjugate region. During these disturbed periods there is a tendency for the evening activity to lag behind that of the morning—as for higher latitude events[3].

GBR (16 kHz) and NAA (17.8 kHz) wave amplification

Further evidence of the importance of man-made v.l.f. emissions in the electron slot has been found in a study of the GBR (Rugby, 16 kHz) and NAA (Maine, 17.8 kHz) wavefields above the ionosphere. Amplification of the NAA signal at 17.8 kHz is commonplace whereas that of the GBR signal is rare. The phenomenon is strongly localised in the invariant longitude sector which includes the South Atlantic magnetic anomaly. This is shown very clearly in Fig. 3 where Ariel III daytime (SZA < 90°) GBR signal intensities measured in the azimuthal sector 225–255°E of N in the period 1967 May 12–August 1 are compared with those observed in the sector 105–135°E of N. Two types of signal, frequency shift keying (FSK) and specially coded, are plotted. The coded signal consists of pure continuous wave transmitted in a 90-s ON, 30-s OFF sequence. It can be seen that only the coded signals in the azimuth-range sector extending over the North Atlantic exhibit amplification. The observations were made using a minimum reading circuit[4], therefore transient amplification events in each observing period of 28 s are excluded. The FSK signal intensities lie close to those expected for propagation in the earth–ionosphere wave-guide with partial transmission through the ionosphere. The most strongly amplified (>10 dB) signals are plotted on the map (N. Atlantic) in Fig. 1. There are two groups; that to the immediate east of the anomaly are events observed at dawn in the period May 16–23 inclusive when the local time and solar zenith angle were ~0600 and ~85° respectively with little magnetic activity ($K_p \simeq 2-$, $\leq 3+$). Further east is a group occurring on June 7 and 8 at local time and zenith angles of ~1600 and ~55° respectively. These observations were preceded by a violent change in the AE index of 1100γ at 0000–0200 UT on June 6. Surprisingly, in none of these GBR events was there evidence of wave amplification in the wavefield observed over the South Atlantic.

The locations of the most intense amplification (>10 dB) events observed for the NAA wavefield in the period 1971 12, December–March 1 are shown in Fig. 1. These all lie in the Southern Hemisphere, slightly to the east of the anomaly, corresponding to amplification of the one-hop whistler mode. The NAA signal throughout this period was almost entirely FSK and was recorded, as for the Ariel III GBR observations, using a minimum reading circuit. An interesting feature of the NAA and GBR one-hop whistler signals, which were observed simultaneously on Ariel IV, was that the GBR signal (FSK) was never amplified in the presence of the more intense NAA signal, however, natural whistlers, at frequencies of 3.2 and 9.6 kHz, did exhibit simultaneous amplification for ~50% of these large amplification events. Apart from a few substorms

Fig. 2 Percentage occurrence of emissions with intensity $>4.8\times10^{-15}$ W m^{-2} Hz^{-1} at 3.2 kHz, annual electric power consumption (in GWh mile^{-2}) and thunderstorm occurrence for the USA $K_p \leq 2+$. – – – – – –, 1967 Summer (May, June, July). – • – • – • – •, 1967 Autumn (August, September, October). Relative thunderstorm occurrence: summer/autumn = 4 (see text).



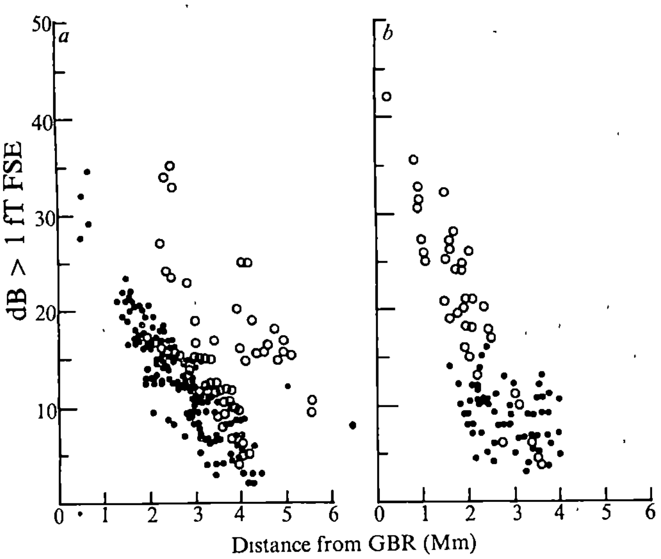


Fig. 3 Daytime amplification of the GBR 16-kHz coded signal over the North Atlantic 1967 May 12–August 1; all K_p; SZA⩽90°. ●, FSK signals; ○, coded (CW) signals. *a*, Azimuthal sector 225–255°E of N; *b*, Azimuthal sector 105–135°E of N

the NAA signal amplification was associated, as for the GBR wavefield, with solar illumination of the ionosphere at dawn. Further studies of the NAA wavefield by D. J. Davies (personal communication) support this conclusion.

Discussion

From the v.l.f. studies reported above, it is clear that man-made transmissions, particularly the power-line harmonics giving rise to the prominent zone of e.l.f./v.l.f. emission above North America, have an important role in the formation of the electron slot in the magnetosphere. Dungey[5] was the first to suggest that v.l.f. signals generated on the surface of the Earth might modify the energetic particle population in the magnetosphere by pitch angle diffusion into the loss cone and various authors (see ref. 6) have studied wave particle interaction in the electron slot region. Amplification of the signals in this sector make them much more effective in causing pitch angle diffusion. More direct evidence for wave amplification at mains harmonics, up to 6 kHz, has been found[7] from ground-based observations in Antarctica. They were able to identify a Canadian aluminium smelting plant as a possible source of some of the signals observed. The signal intensity of the band-limited noise observed on OVI-14 at the magnetic equator lay between 0.03 and 1 m $\gamma\text{Hz}^{-1/2}$ and occurred in the recovery phase of a magnetic storm ($D_{ST} = -120\gamma$). It was sufficient to account for the observed lifetimes of medium and higher energy electrons in the slot. Once the energetic electron population has fallen below the critical flux level indicated by the theory of Kennel and Petschek[8], then precipitation by a self-generated emission becomes impossible and a wave source is required. Lyons, Thorne and Kennel[9] have proposed that the electron slot is formed by high order gyroresonant interaction with plasmaspheric e.l.f. hiss, propagating to lower L shells from just within the plasmapause boundary where it is generated. More detailed correlative studies between wave and particle observations are required to ascertain the relative importance of e.l.f. hiss and power line harmonics (first-order cyclotron resonance). The Ariel III and IV evidence for a strong localisation in v.l.f., but not e.l.f. amplification, over the eastern USA suggests that the latter are more important. Theoretical calculations of energetic electron diffusion by the e.l.f. plasmaspheric hiss and power line harmonics indicate that the latter (in first-order resonance) could make an important contribution to diffusion at larger pitch angles where it was found[9] necessary to invoke higher order resonances for the e.l.f. hiss (Matthews, personal communication). There are also ground-based observations of band-limited hiss (centred on 5 kHz) which occurs during and after great magnetic disturbances at these invariant latitudes[10].

A recent study[11] of the precipitation of energetic electrons from the slot shows that peaked energy spectra, as well as the more normal exponential energy spectra of such electrons, may occur. This peak in the energy spectrum moves to higher energies with decreasing L in such a way that it could be attributed to a first-order cyclotron interaction at a single wave frequency over the range of L sampled. This, of course, is what we might expect to observe for the NAA and/or GBR signal. For the daytime model magnetosphere of Alexander[12] the parallel electron energies for first-order cyclotron resonance at the equator vary from 1 MeV at $L = 1.8$ to 6 keV at $L = 3.0$. Imhof *et al.*[11] ascribe the majority of their peaks to first-order resonance at 10 kHz and below (based on the model magnetosphere used) however, lack of data on the actual cold plasma distribution at the time of observation as well as the possibility of a wave–particle interaction at a distance from the equator, make it possible that a higher frequency was involved. The parallel energy, E_R, of the resonant electron is given by

$$E_R = E_c \frac{\omega_H}{\omega}\left(1-\frac{\omega}{\omega_H}\right)^3$$

where $E_c = B^2/2\mu_0 N$ is the magnetic energy per particle, $\omega_H = \frac{eB}{m}$ is the electron gyrofrequency (rad s^{-1}), $\omega = 2\pi f$, B is the local magnetic field, N the local electron density, e the electronic charge, m the electron mass and f the wave frequency (SI units). E_R is clearly very dependent on the value of B.

It has been known for some time (see refs 13–16) that the wave–particle interaction for a ground-based transmitter is a strong function of the duration of the transmitter pulse which causes phase-bunching of the electrons. The preferential amplification of the long duration coded GBR signal, in what is probably the two-hop whistler mode, provides a good example of this. The localisation of the GBR and NAA amplification, slightly to the east of the anomaly, comes from the increase in loss cone and pitch angle anisotropy[6] of electrons drifting eastwards through the anomaly. Also in this region field aligned structure will help by ducting the whistler wave along the same path as the interacting electrons. The maximum at dawn may be attributed to the decrease in E_c corresponding to an increase in electron density, N, as the cold plasma flows up the field lines from the ionosphere. Thus E_R is decreased and lower energy electrons, which are normally more numerous, drift eastwards into a region where cyclotron resonance becomes possible.

In conclusion, the power line harmonics and other man-made transmissions may, by first-order resonance with the energetic electrons, make a significant contribution to the electron slot between the radiation belts. Thunderstorms over North America also produce e.l.f./v.l.f. wavefields of significant intensity.

Received November 24, 1975; accepted February 19, 1976.

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Protein-folding dynamics

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In a discussion of the dynamics of protein folding two limiting models (random-search nucleation and chain propagation, diffusion–collision) are considered. It is suggested that the latter may have the dominant role in many proteins.

THE problem of the folding of a globular protein to its native structure has two parts[1]. The first is the static aspect concerned with the elements in the amino acid sequence that provide the information; the second deals with the dynamics of the folding process itself. Clearly, the answer to the first part of the problem is involved in the second (in particular, the stabilities of intermediate structures may be important in selecting the folding paths) and conversely, it is possible, though less likely, that the second affects the first (that is, that the nature of the folding process results in a non-equilibrium structure). Available data indicate that proteins *in vitro* can fold up to their native structure in times from tenths of seconds to minutes in the absence of S–S bridges[2]; formation of S–S bridges coupled to refolding tends to take longer. This is to be contrasted with the long times required to find the native conformation in a random search of all possible structures (for example, in the simplest model, such a random search would take on the order of 10^{50} yr for a protein consisting of 100 amino acids). Thus, protein folding must make use of more sophisticated search procedures, which, in one way or another, divide a protein molecule into parts such that the information contained in the sequence of each one can be used independently. The simplest solution would be that a protein consisting of 100 amino acids is composed of ten or so approximately equal parts, each of which finds its own unique stable form, and that these parts then come together to form the complete native structure. But it seems from studies of peptides in solution[3–8], as well as approximate energy calculations[9], that most sequences short enough to be searched through rapidly do not have a folded structure that is sufficiently stable. Thus, alternatives to this simple idea must be examined. Two possible limiting mechanisms for globular protein folding are discussed further below; they are referred to as the random-search nucleation and chain-propagation model and the diffusion–collision model. The latter mechanism is suggested to be more important than the former, though the folding of any particular protein may involve some elements of both limits.

Random-search nucleation and chain propagation

The model utilises a random-search nucleation step as the start of the folding process followed by chain propagation to obtain the native structure. The basic elements of such a mechanism are that first, a portion of the protein is small enough for a random search and yet stable enough to serve as a nucleation unit, and second, that once this unit has the native structure, it makes possible a sequential, essentially independent folding of subsequent amino acids. Such a mechanism was outlined by Wetlaufer[10], and earlier by Levinthal[11,12]. The process in its simplest version can be formulated by analogy with the treatment of helical dimer formation involving complementary oligonucleotides[13,14] or helix formation in a polypeptide with only a single nucleation site[15,16]. Tsong *et al.*[17,18] have provided a kinetic model for such sequential protein folding and given kinetic data[19,20] related to it. The chain-propagation steps are assumed to be analogous to the addition of units in helix formation in polypeptides and, therefore, very fast (that is, of the order of 10^{-6}–10^{-8} s). Concerning the nucleation step, it is more difficult to make a general statement, although a random search of the conformational alternatives of a minimal nucleus composed of approximately fifteen amino acids would require of the order of milliseconds.

Diffusion–collision

An alternative limiting possibility, the diffusion–collision model, considers the protein molecule to be divided into several parts (microdomains), each short enough for all conformational alternatives to be searched through rapidly. This condition implies that the native secondary structure of each such microdomain, though accessible by random events, is not stable. Consequently, several (two or more) of them have to diffuse together and collide in order to coalesce into a structural entity with the native conformation.

Anfinsen[21] has discussed the possibility that portions of a protein chain can "flicker" in and out of their native format and serve as nucleation centres coalescing through non-covalent interactions to give the native protein structure; this statement is suggestive of the diffusion–collision model.

To examine the factors determining the kinetics of the model, we consider an example involving two microdomains; this could represent a protein fragment composed of only two parts, or more generally, a portion of an entire protein. Each of the microdomains can be described as being in equilibrium between the native secondary structure and the unfolded random-coil structure, which includes the rest of the available conformational space. If the microdomains formed helices in the native state (as in myoglobin) the helix, random-coil transition would be involved. We assume here that coalescence can occur only if both the partners have the native structure when they collide and that the secondary structural transitions are fast relative to other processes. If the two connected units are regarded as diffusing together from a finite distance to form a stabilised entity with the native conformation, an order of magnitude for the time τ required for the coalescence can be obtained from the radial diffusion behaviour of spherical units. The result is (details will be given elsewhere)

$$\tau \simeq \frac{1}{\beta}\left(\frac{l\Delta V}{DA}\right) \tag{1}$$

where ΔV is the volume of the finite diffusion space (the space between two concentric spherical shells); A is the target surface area (the area of the inner shell) whose radius is determined by the sizes of the peptide units; D is the diffusion coefficient and l is a characteristic length (the average of the inner and outer shell radii, the latter being determined by the maximum distance of separation of the two units which are part of the same protein chain). The quantity β accounts for the fact that only a fraction of the microdomains have the native secondary structure when they collide. In a detailed theory, the various parameters involved (that is, ΔV, A, l) would have a direct physical significance, but in this heuristic discussion they are phenomenological in character and only order of magnitude values can be given. With $D \sim 1 \times 10^{-6}$ cm^2 s^{-1}, a value appropriate for spherical particles with the dimensions of protein fragments, and with the outer shell radius twice the inner one and equal to 2×10^{-7} cm (20 Å), equation (1) yields $\tau\sim\beta^{-1}[10^{-7}\text{–}10^{-8}]$s. To evaluate β, an equilibrium model can be used for simplicity; that is, $\beta=K_1K_2/(1+K_1)(1+K_2)$ where K_1 and K_2 are the (native)/(random coil) equilibrium constants for the two separated units. This expression ignores the possibility that β could vary as the partners approach and that details of the kinetics might modify the equilibrium result. From the above formulae for τ and β, it follows that for values of K_1 and K_2 equal to 10^{-3}–10^{-4}, orders of magnitude equal to the estimated equilibrium constant for helix nucleation in helix–coil transitions[22] and the native–random coil equilibrium in protein fragments[3–8,23], the folding time for a pair of units is of the order of 0.01 and 10 s. If several units have to come together at one time to form a stable entity and each one has a K_i value less than 10^{-3}, the folding time would be inordinately long. Consequently, a stepwise folding by this mechanism seems most likely; for example, two units coalesce to form a slightly more stable entity which in turn collides with a third entity, and so on. In this way the rate of the process would increase as the folding advanced, as expected for a cooperative transition.

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Variation of τ with temperature is expected due to both D and β. The Einstein–Stokes expression for D approximately doubles in size between 10 °C and 40 °C. The dependence of β on temperature is difficult to estimate since it is determined by a balance between enthalpic and entropic (for example, hydrophobic) terms. Some temperature variation has been observed in the renaturation rate of acidified staphylococcal nuclease[24]. The results are not, however, sufficiently detailed to relate to the model discussed here.

Several refinements would have to be introduced into the model to make calculations of more than order-of-magnitude validity. These include a more detailed consideration of the size, shape and connections of the colliding segments, the possibility of the existence of an activation barrier to coalescence, the requirement for rotational as well as translational motion to obtain the correct position for a stable interaction, and the consideration of diffusion in the presence of a folding/unfolding process with the same time scale. Most of these effects are expected to increase the time required, though changes by several orders of magnitude seem unlikely except for the significant activation barrier.

Implications of the models

Two limiting mechanisms that could be involved in the folding of proteins to their native structure have been discussed briefly and their dynamics considered. The true folding process of a real protein may well involve elements of both extremes. Some amino acids would undoubtedly take up the native format in a sequential, rather fast (μs) manner whether the primary process were a random-search nucleation or a diffusion–collision step or a combination of the two. It seems, however, that folding an entire protein of 100 or more amino acids by a single random-search nucleation plus chain-propagation mechanism is not feasible. The difficulty is that rapid propagation after nucleation requires that the laying down of each successive amino acid or small group of amino acids be essentially independent of the conformation of the rest of the unfolded molecule. Although this may be a good approximation for helix formation, it seems unlikely for the complicated structure of globular proteins. If each propagation step requires a search through a significant fraction of the conformations of the uncoiled portion of the protein chain, the basic folding problem, albeit in a slightly reduced form, remains unsolved after nucleation. It has been noted in analyses of structural data that many larger proteins are composed of domains, each of which could fold almost independently[6]. Since these domains are composed of 50–150 amino acids, they are too large to serve as primary nucleation centres; instead they each raise the same folding problem as the entire molecule.

In contrast to the difficulties that are found with the random-search chain-propagation model, available structural and other data suggest that the diffusion–collision model can serve as the basis for a mechanism of protein folding. Several recent studies have shown that globular proteins have about one-third of their residues involved in chain-reversal turns[25–27]. Since each turn is normally composed of four residues (by the criteria used in the analyses), this result implies that a protein is on average composed of segments eight to ten residues between turns. Thus, it seems natural to suggest that these segments form the primary diffusing units in the diffusion–collision mechanism. The entire folding process would, as described above, involve a series of such diffusion–collision steps. These might have to follow a unique order to yield the native structure (single correct pathway); alternatively, different sequences of diffusion–collision steps might be possible. Further, particularly in the larger proteins, there may be several regions (the domains) that are individually fixed in their native structure by a set of diffusion–collision steps and finally come together to form the completed native protein molecule.

The diffusion–collision mechanism would be most effective if the individual segments (the diffusing units or microdomains) were relatively unlikely to bend significantly; that is, that they can be regarded as statistically rigid rods in the diffusion process so as to minimise entanglement problems. Empirical correlations tend to support this view[25–27], as does the model calculation of Levitt and Warshel[28]. Some regions which have a very well defined secondary structure (for example, α helices) could support more residues per segment; significant deviations from the average of eight to ten residues do occur, although a detailed correlation with the folding mechanism has not been made.

The motion emphasised here is essentially diffusive, and the attractive forces that stabilise the coalesced microdomains are short range; that is, until two groups are sufficiently close to prevent water molecules from being between them, no hydrophobic attraction can exist. Moreover, any directing forces provided by the connecting elements are likely to be damped by hydrodynamic effects. Once the segments have diffused together, the balance between hydrophobic attraction and van der Waals' repulsion will determine whether or not coalescence can occur in a given collision. This requires that segments have the correct secondary structure (including the side-chain orientation)[29]; otherwise a significant repulsive barrier would be expected. Consequently, the parameter β, which determines the fraction of the time that the correct secondary structure exists, has a fundamental role in the effective time constant of the diffusion–collision mechanism (equation (1)). It would be very useful to have experimental measurements or theoretical estimates of β for protein fragments that are likely candidates for the microdomains involved in the diffusion–collision process. These might be pairs ofhelices, pairs of peptide chain segments that can form a β sheet, or an α-helix plus β-sheet structure (ref. 30 and references therein); in the present context, a β sheet is regarded as an element of tertiary structure because it is formed by the coming together of two elements, each with the appropriate extended secondary structure. Such fragments might be made available for study either by cleavage of the native protein or by direct synthesis. Comparisons can be made also with the available equilibrium constants for the (native)/(random coil) transition of complete proteins[31]. Kinetic measurements for the renaturation of fragment-type systems are important as well; some data are available for the *Staphylococcus* nuclease peptides[32]. Possible model systems include glucagon (which has a random coil structure in dilute solution, but at high concentration forms a trimer composed mainly of α helices)[6–8], and the polypeptides that form β-sheet networks[33].

Incorrectly folded intermediates have been suggested on the basis of a kinetic analysis by Ikai and Tanford[34]. Creighton[35] has trapped intermediates with incorrect disulphide bonds in his study of the folding of the basic pancreatic trypsin inhibitor; both the correct and incorrect early intermediates (one disulphide bond) seem to be correlated with stabilising secondary structural elements of the type mentioned above[25–27,30]. Such incorrect folding might be expected if the diffusion–collision mechanism has an important role; for example, segments that are not together in the native structure could collide and form intermediates which are relatively unstable because of improper non-bonded contacts.

Two theoretical attempts[28,36] to fold a protein have been made as a means of investigating the static part of the problem. One of these, by Ptitsyn and Rashin[36], considered myoglobin as composed of stable helices and, on the basis of a simple model for hydrophobic interactions between the helices, compared different paths to the most likely folded structure, a rough approximation to the native conformation. This type of static analysis is certainly compatible with the diffusion–collision mechanism; in a corresponding kinetic model, the helix–coil equilibria of the different helices would have to be taken into account. Levitt and Warshel[28] have tried to obtain the native structure of the basic pancreatic trypsin inhibitor by energy minimisation. They began with a simplified model of the extended polypeptide chain that can bend easily at only a few amino acids. The protein is thereby divided into segments that simplify the folding process as in the diffusion–collision mechanism. An important additional element in their calculation is that the assumed balance between attractive and repulsive forces is such that the colliding segments do not need to have the correct secondary structure for coalescence to occur. If this were valid for the actual folding process, the kinetic behaviour would be significantly different from that described above, since the parameter β would not appear in equation (1). There would result a rapid diffusion step leading to an approximate structure, followed by a slower process involving local readjustments to the native structure. Most likely, the situation in proteins is intermediate, with some probability of coalescence even when the colliding segments have an only approximately correct secondary structure.

Many of the data used in published discussions of protein folding are obtained in studies of unfolding[2]. This raises the question of whether the pathways for the two processes are the same. Since diffusion of polypeptide segments over the distances involved requires less than a microsecond, it is hard to account for the observed unfolding times, which are of the order of seconds, by assuming a simple reversal of the folding process. Consequently, it seems necessary to introduce a slow random event (for example, nucleation of open regions) in the unfolding pathway that is different from that in folding. An alternative possibility, which has not been considered in detail here, is that both processes are limited by a high activation barrier involving the same intermediates.

The analysis given here shows that the information required for a complete description of the dynamics of protein folding is not yet available. We hope, however, that our discussion of different limiting mechanisms will stimulate others to perform the experiments necessary to clarify the processes involved.

Helpful discussions with Drs S. C. Harrison and J. R. Knowles are gratefully acknowledged. This work was supported in part by grants from the US National Science Foundation and the National Institutes of Health.

Received December 4, 1975, accepted February 3, 1976.

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Mechanism localisation and control of restriction cleavage of phage T4 and λ chromosomes *in vivo*

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The primary action of restriction endonuclease, cleaving infecting DNA, has been demonstrated in vivo. *This primary cleavage is followed rapidly by hydrolysis of the cleaved DNA at its newly exposed termini. Infecting viruses can inactivate cytoplasmic and membrane restriction endonucleases to prevent cleavage of unmodified DNA replicas.*

THE utility of restriction endonucleases as a tool in biological research and their potential in genetic engineering have aroused much interest in these enzymes[1,2]. Considerable information exists on the mechanism of action of restriction enzymes *in vitro*[3-5]. Much less is known about their function within cells. Recognition *in vitro* is somewhat ambiguous[6] and in fact some chromosomes which are cleaved by isolated restriction endonucleases *in vitro* are not restricted by bacteria containing these endonucleases *in vivo*[7,8]. Restriction endonucleases normally recognise a specific short nucleotide sequence in double-stranded DNA. There are two major classes of purified restriction endonucleases, defined as specific (class II) and nonspecific (class I), depending on their cleavage specificity *in vitro*[2]. Class II enzymes cleave the DNA at the restriction site by opposite or staggered nicks[9-13]. This distinction can be reduced by altering incubation conditions[6]. In addition, single-stranded DNA can be cleaved[14] in certain conditions. Class I enzymes can cleave at any place up to a few hundred nucleotides away from the recognition site. We wonder which of these variations reflect the variety of mechanisms of action of these enzymes within the

Table 1 Restriction and breakdown of T4 glu$^-$ DNA and T4 2$^-$ DNA in various hosts

E. coli strain	*rgl*	exo V	exo I	T4 phage	Plating efficiency	% TCA-soluble DNA
K	−	+	+	glu$^-$	1	3
K	−	+	+	2$^-$	1×10^{-4}	40
B	+	+	+	glu$^-$	1×10^{-5}	42
B	+	+	+	2$^-$	5×10^{-6}	48
W3110	+	−	+	glu$^-$	2×10^{-4}	8
W3110	+	−	+	2$^-$	0.7	8
JC7623	+	−	−	glu$^-$	2×10^{-4}	5
JC7623	+	−	−	2$^-$	1	6

Standard phage techniques were those of Adams[31] and other techniques and strains used were described by Silverstein and Goldberg[29,30]. DNA breakdown is determined as the TCA-soluble fraction of DNA. Glu$^-$ phage were T4αgt^{57}βgt^{14} grown on *E. coli* K *rgl*$^-$, 2$^-$ were T4 am N51 grown on *E. coli* B. The data given are not corrected for absorption which was 85–90% for both 2$^-$ and glu$^-$ phage.

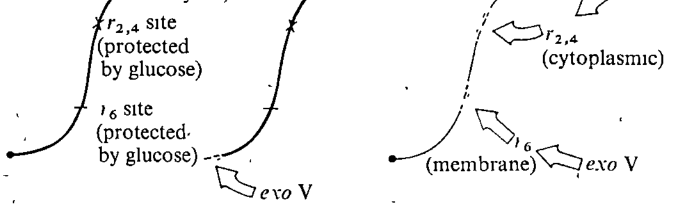
cell and which are merely artefacts of the *in vitro* environment.

Recognition sites in the DNA may be modified specifically (for example, methylation of *E. coli* DNA and λ DNA[15,16] or glucosylation of T-even DNA[17-19]), rendering the DNA insensitive to the appropriate restriction enzyme. *In vitro*, the action of restriction enzyme is usually studied by observing the cleavage of an intact viral chromosome into large fragments. For example, phage λ DNA is cleaved *in vitro* at five sites by fi$^+$ R-factor-coded restriction endonuclease (endo R. *Eco*RI[20]). *In vivo*, the cleavage by restriction endonucleases is followed by extensive hydrolysis by nonspecific nucleases[21-25], resulting finally in acid-soluble fragments[12,26]. We call this hydrolysis DNA breakdown, to distinguish it from the initial cleavage. Breakdown of unmodified λ DNA in *E. coli* K depends on the function of the bacterial *rec* BC locus. Growth of unmodified phage is restricted in *rec* BC$^-$ hosts even though DNA breakdown does not occur[27]. In considering the possible biological mechanism of action of restriction enzymes in the cell, we were surprised that large DNA fragments have never been demonstrated *in vivo* as primary products of restriction endonuclease cleavage. In this report we try to clarify the relationship between the initial cleavage by restriction endonucleases and the subsequent breakdown of the cleaved fragments by exonuclease V (exo V is specified genetically by *rec* BC)[28] *in vivo*. Using *rec* BC$^-$ bacteria, we were able to prevent degradation of the fragments cleaved from the unmodified T4 or λ phage chromosome by the cellular restriction endocucleases. Therefore we were able to demonstrate that these large fragments are intermediates in the restriction process *in vivo*, as postulated after *in vitro* experiments. We were also able to show that different restriction endonucleases which are thought to be localised in the cytoplasm or in the membrane can be inactivated in different ways by the infecting phage.

Role of cleavage and breakdown in restriction of T4

A phage T4 amber mutant in gene 2 produces particles of normal appearance in non-permissive Su$^-$ hosts. Though they adsorb and eject DNA normally, they do not produce progeny on Su$^-$ or Su$^+$ bacteria unless the bacteria lack *exo* V[29,30]. Table 1 compares the restriction (plating efficiency) of phage particles defective in gene 2 (2$^-$) and non-glucosylated (glu$^-$) phage and the breakdown (% of trichloroacetic acid (TCA)-soluble DNA) of their DNA after infection. In the presence of exo V both 2$^-$ and glu$^-$ infecting chromosomes are broken down, but in the absence of exo V they are not. In the presence of *rgl* (the restriction systems for non-glucosylated T-even phages) growth of 2$^-$ phage is not restricted, whereas growth of glu$^-$ phage is. In *rgl*$^-$ hosts, with or without exo V, glu$^-$ DNA is not broken down nor are glu$^-$ phages restricted.

Fig. 1 A model for the mechanism of restriction of 2$^-$ and glu$^-$ T4 phage by endonucleolytic cleavage and exonucleolytic breakdown of DNA. Thick line, glucosylated DNA; thin line, non-glucosylated DNA.



These findings can be explained by the model illustrated in Fig. 1. T4 gene 2 specifies a protein (P2) which seems to protect the end(s) of the entering phage genome from exo V. Modification of the DNA by glucosylation protects the entering genome from the *rgl* endonucleases. In the absence of P2, exo V would digest the entering DNA exonucleolytically. This action would thus be directly responsible for both the breakdown of part of the genome and the consequent restriction of the phage. The presence of P2 on the unmodified genome could protect the natural termini from exo V, but could not protect newly exposed ends created by endonucleolytic action of the *rgl* systems. Thus, as Table 1 shows, restriction of T4 glu$^-$ phage would be a direct result of cleavage of the genome by the *rgl* systems, whereas breakdown would be a secondary result of exo V action on the newly formed unprotected ends.

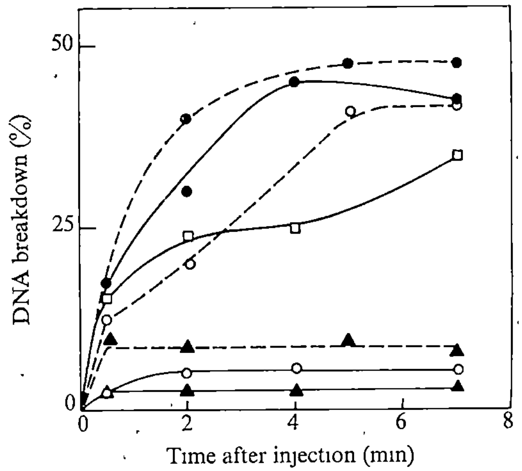


Fig. 2 Kinetics of breakdown of T4 glu$^-$ DNA and T4 2. Su$^-$ DNA in various hosts Experimental details are as described in Table 1. ●—●, glu$^-$ T4 in *rgl*$^+$ exo V$^+$ exo I$^+$ (B); ▲—▲, glu$^-$ T4 in *rgl*$^-$ exo V$^+$ exo I$^+$ (K *rgl*$^-$); ○—○, glu$^-$ T4 in *rgl*$^+$ exo V$^-$ exo I$^-$ (JC7623); □—□, glu$^-$ T4 in *rgl*$^+$ exo V$^+$ exo I$^-$ (W3110 sbcB); ●- - -●, T4 2 Su$^-$ in *rgl*$^+$ exo V$^+$ exo I$^+$ (B); ▲- - -▲, T4 2.Su$^-$ in *rgl*$^+$ exo V$^-$ exo I$^-$ (JC 7623); ○- -○, T4 2.Su$^-$ in *rgl*$^+$ exo V$^+$ exo I$^-$ (W3110 sbcB).

According to this model, it should be possible to demonstrate *in vivo* the initial cleavage products of unmodified DNA by infection of cells lacking exo V. To demonstrate such fragments we had first to determine the proper strains and appropriate time after infection to extract the DNA. Figure 2 shows that when exo V cannot digest the DNA, there is little acid-soluble material produced up to 7 min. This is the case even up to 25 min (unpublished). In cases where breakdown occurs it is usually complete by 5 min after infection. When

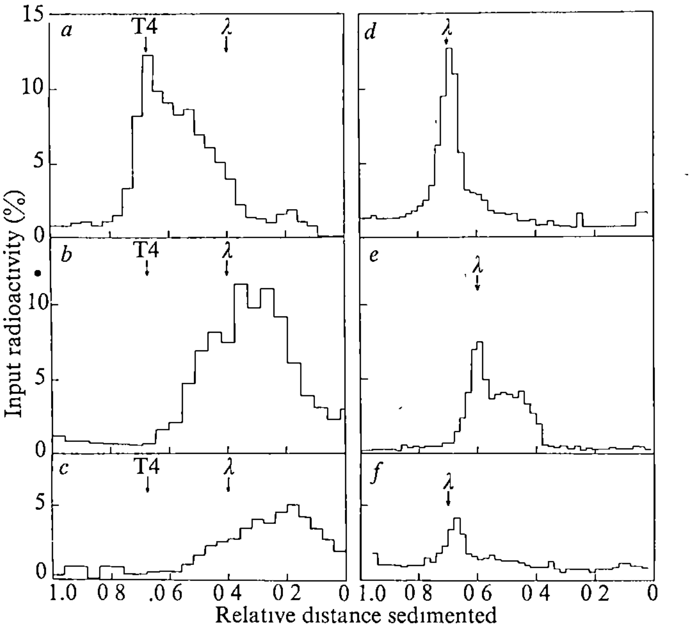


Fig. 3 Demonstration of primary cleavage products of *rgl* and r_K restriction *in vivo* *a*, *b* and *c*: T4, *rgl* restriction. Cells (5×10^8 per ml) were infected at 37 °C with two or three ^{32}P-glu$^-$ T4 phage each. After 5 min aeration at 37 °C cells were lysed and 0.2-ml samples were layered on to 4.5 ml 5%–20% alkaline sucrose gradients[32]. Marker phage (^{3}H-λ) was added to the infected cell lysate after addition of sarkosyl The DNA was sedimented for 100 min at 147,000*g* at 20 °C, fractionated by dripping on to Whatman scintillation pads, and washed with cold TCA. The 5% cold TCA removed acid-soluble nucleotides and therefore some of the graphs show less than 100% recovery. The calculated position[33] of T4 DNA shown in each graph was checked experimentally on several occasions. *a*, *rgl*$^-$ exo V$^+$ exo I$^+$ (K rgl$^-$); *b*, *rgl*$^+$ exo V$^-$ exo I$^-$ (JC 7623); *c*, *rgl*$^+$ exo V$^+$ exo I$^+$ (B). *d*, *e* and *f*: λ, r_K restriction. Cells (5×10^8 per ml in 0 01 M Tris buffer, *p*H 7.4, 0.001M $MgSO_4$) were infected with two or three ^{32}P-λC_{I857}· C_{600} r_K^- m_K^- per cell. Cells and phage were prepared as described before[34]. After 5 min of aeration at 37 °C cells were lysed and analysed in alkaline gradients as described above except they were run at 192,000*g* for 120 min. Adsorption of λ was 85–90% at 5 min after infection for all cells. *d*, r_K^- m_K^- exo V$^+$ (C600); *e*, r_K^+ m_K^+ exo V$^-$ (JC 7623); *f*, r_K^+ m_K^+ exo V$^+$ (QL).

exo V digests DNA in the absence of exo I, the production of acid-soluble fragments is delayed to some extent (Fig. 2). Thus the presence of exo I makes, at most, a transient difference in acid solubility and does not affect cleavage by *rgl*.

The products of cleavage and subsequent breakdown were analysed by sucrose gradient centrifugation (Figs 3 and 4). In the absence of exo V the *rgl* systems cut each DNA strand in only a few places and there is but little breakdown of DNA to small acid-soluble or insoluble fragments (Fig. 3*b*). DNA strands from non-glucosylated T4, are intact after infection of a host which contains exo V but lacks the *rgl* systems (Fig. 3*a*). Hosts with both exo V and *rgl* systems yield DNA strands which are much shorter on the average than in exo V$^-$ *rgl*$^+$ hosts, and much of the DNA is lost when the filters are washed with TCA since it is TCA-soluble (Fig. 3*c*). Neutral gradients give results consistent with this interpretation (data not shown), since on *rgl*$^+$ exo V$^-$ hosts the entering DNA is cleaved and sediments as a few peaks, the amplitude of which is greatly reduced in *rgl*$^+$ exo V$^+$ hosts. Thus we have been able to demonstrate the *in vivo rgl* cleavage products of unmodified T4 genomes. This cleavage prevents T4 growth even in the absence of breakdown by exo V (Table 1).

The model in Fig. 1 predicts a very different result for the degradation of the 2$^-$ genome. If restriction is a direct result of exonucleolytic attack, the large DNA fragments should get progressively shorter, and the small single-stranded excised pieces should be produced directly without intermediate sized fragments. Figure 4 shows the time course of degradation of parental 2$^-$ DNA in *rgl*$^+$ exo V$^+$ and *rgl*$^+$ exo V$^-$ hosts. The DNA molecules are gradually shortened, and the excised single-stranded fragments are converted directly to small acid-soluble or insoluble pieces in exo V$^+$ cells. This is consistent with the action *in vitro* of exo V, which degrades double-stranded DNA directly into single-stranded fragments one to several hundred nucleotides long[35]. Since 2$^-$ DNA is fully glucosylated it is not cleaved by the *rgl* system. Figure 4 shows a small amount of short acid-insoluble oligonucleotides sedimenting between 0 and 15% from the top of the gradient. The amount of this material is roughly constant from 1 to 4 min after infection while the total amount of breakdown products as measured by acid solubility rises rapidly (30%, 40%, 45% and 48% in 1, 2, 3 and 4 min, respectively). Thus it seems that exo V, *in vivo*, hydrolyses DNA into oligonucleotides up to 500–600 units long (remaining at the top of the gradients), which may well be the initial products of degradation. These are then further degraded to acid-soluble material by exo V, as has been shown *in vitro*[35]. This secondary degradation to mononucleotides may be aided by exo I which can rapidly hydrolyse short single-stranded DNA fragments of glucosylated as well as non-glucosylated DNA to mononucleotides. This idea is consistent with our findings (Fig. 2) that in exo V$^+$ exo I$^-$ cells, the breakdown of glu$^-$ and 2$^-$ DNA is slower than in exo V$^+$ exo I$^+$ strains. The final ratio of acid-soluble to insoluble

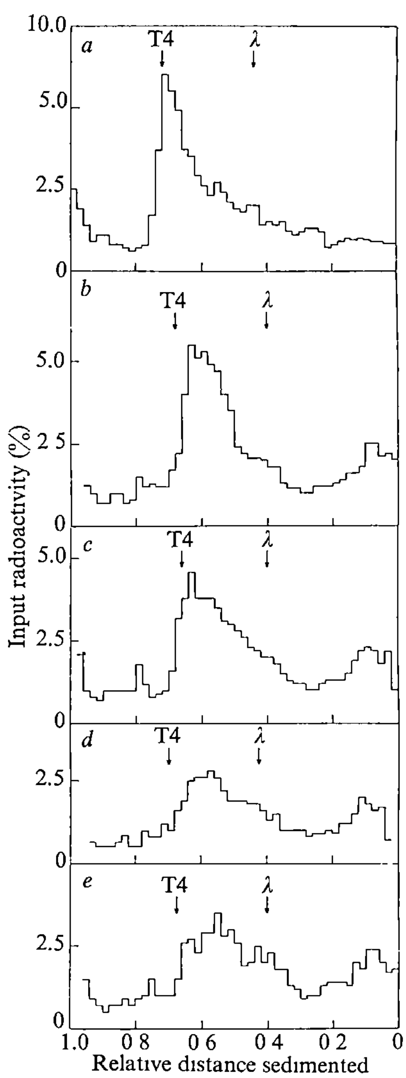


Fig. 4 Kinetics of digestion of T4 2$^-$ DNA *in vivo*. Experimental details were as described in Fig. 3. Aliquots of infected cells were lysed at the appropriate times and 0.2-ml samples were layered on the alkaline sucrose gradients *a*, exo V$^-$ exo I$^-$ (JC 7623) cells lysed 5 min after infection. *b*, *c*, *d*, and *e*, exo V$^+$ exo I$^+$ (B) cells lysed 1, 2, 3 and 4 min, respectively, after infection

material may be due in part to reincorporation of acid-soluble products into acid-insoluble material.

Simmon and Lederberg[27] showed that breakdown of unmodified λ DNA *in vivo* is aided by exo V in restricting hosts, and is independent of other nucleases, like exo I. Based on our data on glu^- T4 restriction, our model (Fig. 1) explains the mechanism of restriction of T4 *in vivo* independently of exo V. According to this model, unmodified λ should be restricted even in exo V^- hosts. Restriction of growth of unmodified λ has already been shown[27]. In restricting exo V^+ cells, at low multiplicities of infection, unmodified λ DNA is broken down to acid-soluble nucleotides (Fig. 3*f*). Only about 10% of the DNA sediments along with the marker DNA, and no high molecular weight intermediates are found in these conditions. Analysis of restriction products in similar conditions in a restricting exo V^- host is shown in Fig. 3*e*. About 75–80% of the λ strands are cut into large fragments of 0.4–0.9 the size of λ. This agrees with the *in vitro* results[9,12] first shown by Meselson and Yuan. The rest of the DNA strands cosediment with whole λ marker strands. Only about 5% of the DNA is degraded to acid-soluble fragments. This indicates that the absence of large intermediates in exo V^+ cells is due to the secondary action of exo V. The efficiency of restriction is more or less the same in restricting hosts whether or not exo V is present.

Localisation of r_6 and $r_{2,4}$

Revel[36] showed that the *rgl* function is controlled by two genes: r_6 which restricts all glu^- T-even phages, and $r_{2,4}$ which restricts only glu^- T2 and T4. Fukasawa[22] proposed that the *rgl* system is a membrane function since modified (glu^+) parental DNA could escape restriction in an rgl^+ UDPG pyrophosphorylase$^-$ host allowing the unmodified (glu^-) progeny DNA to replicate and mature. Fleischman and Richardson[37], however, on the basis of *in vitro* experiments showed that *rgl* acts on replicating DNA. Though their data seem to support a cytoplasmic site for $r_{2,4}$, they point out it may nevertheless be membrane bound. Hewlett and Mathews[38] reinforced the proposal of Fukasawa by showing that superinfecting glu^- T6 is not restricted after T4 *imm*$^-$ primary infection. (The *imm*$^-$ mutation prevents exclusion and breakdown of superinfecting T4 chromosomes). They suggest that the mere attachment of the primary phage inhibits *rgl* functions since the successful passage of the superinfecting glu^- T6 does not depend on primary phage protein synthesis. Silverstein and Goldberg[30] found that successful superinfection of glu^- T4, in contrast to glu^- T6, requires protein synthesis by the primary phage. They suggested that, although r_6 may indeed be localised in the membrane, as Hewlett and Mathews[38] concluded, $r_{2,4}$ is probably cytoplasmic, as suggested by the experiments of Fleischman and Richardson[37]. On the basis of these experiments and those reported below, we suggest that r_6 is a membrane function whereas $r_{2,4}$ is probably cytoplasmic.

Table 2 shows that superinfecting glu^- DNA is broken down in $r_{2,4}{}^+$ cells only when expression of the primary infecting phage is prevented. Expression was prevented either by addition of chloramphenicol before primary infection, or by using heavily ultraviolet-irradiated primary infecting phage, which cannot express many genetic functions after infection (for

Table 2 Localisation of restriction functions $r_{2,4}$ and r_6

Bacterial strains	Chloramphenicol	Primary phage	Superinfecting phage	% DNA breakdown of superinfecting phage
$r_6{}^-r_{2,4}{}^-$	−	T4*imm*$^-$	T4glu^-	3
		T4*imm*$^-$	T6glu^-	4
	+	T4*imm*$^-$	T4glu^-	3
		T4*imm*$^-$	T6glu^-	3
	−	UV.T4	T4glu^-	14
		UV.T4	T6glu^-	9
$r_6{}^+r_{2,4}{}^-$	−	T4*imm*$^-$	T4glu^-	4
		T4*imm*$^-$	T6glu^-	2
	+	T4*imm*$^-$	T4glu^-	3
		T4*imm*$^-$	T6glu^-	8
	−	UV.T4	T4glu^-	16
		UV.T4	T6glu^-	6
$r_6{}^-r_{2,4}{}^+$	−	T4*imm*$^-$	T4glu^-	7
		T4*imm*$^-$	T6glu^-	3
	+	T4*imm*$^-$	T4glu^-	28
		T4*imm*$^-$	T6glu^-	3
	−	UV.T4	T4glu^-	43
		UV.T4	T6glu^-	12
$r_6{}^+r_{2,4}{}^+$	−	T4*imm*$^-$	T4glu^-	5
		T4*imm*$^-$	T6glu^-	3
	+	T4*imm*$^-$	T4glu^-	35
		T4*imm*$^-$	T6glu^-	3
	−	UV.T4	T4glu^-	35
		UV.T4	T6glu^-	9

The log phase cultures were collected and resuspended at a cell density of 5×10^8 cells per ml in broth and three T4 *imm*2 phage were added per bacterium After 5 min two or three ^{32}P-glu^- T4 were added per bacterium. Chloramphenicol was added (100 μg ml^{-1}) when required 3 min before addition of primary phage. Adsorption and DNA breakdown were assayed 5 min after addition of superinfecting phage Wild-type T4 phage (10^{11} per ml) were irradiated in washing fluid[29] for 10 min to a survival of <0.001% These UV T4, killed cells at an efficiency of 90% of unirradiated phage. UV T4 particles were used for primary infection at a multiplicity of 4–5 ^{32}P-T6αgt^{am10am16} grown on K *rgl*$^-$ Su^- were used for superinfection experiments.

example, immunity to superinfection, T4-induced endonuclease[39] and anti-exo V, Table 2). Superinfecting glu$^-$ T6 DNA, however, is protected from breakdown in r_6^+ (with or without $r_{2,4}$) cells by the primary infecting phage, even when expression of the primary phage is prevented by chloramphenicol or ultraviolet irradiation.

Controls of restriction systems

The breakdown of superinfecting glu$^-$ DNA by exo V in an $r_{2,4}^+$ exo V$^+$ cell, in the absence of primary phage expression, implies previous cleavage of the superinfecting DNA by an *rgl* enzyme (Fig. 1). The lack of breakdown of the superinfecting glu$^-$DNA in an $r_{2,4}^+$ exo V$^+$ cell, after expression of the primary phage, is to be expected (whether or not the *rgl* system is working) as the result of the expression of an exo V inactivating activity by the primary infecting phage[40,41]. We have shown that the glu$^-$ superinfecting DNA is not cleaved in $r_{2,4}^+$ cells[42]. Therefore the primary infecting phage must code for an anti-$r_{2,4}$ activity which inactivates $r_{2,4}$ after infection. Thus, the reason that unmodified progeny T-even DNA can replicate from a modified parental template and mature in $r_6^+r_{2,4}^+$ hosts (where unmodified parental DNA is normally restricted) is because the early interaction of the infecting phage particle with the host surface inactivates r_6, and subsequent expression of the unrestricted parental genomes leads to a protein which prevents $r_{2,4}$ action on the unmodified progeny DNA.

It is interesting in this respect that unmodified λ is also not restricted by the cytoplasmic r_K in the presence of the sbcA mutation[27]. It would be interesting to know if the sbcA mutation leads to inactivation of r_K or possibly whether it prevents the action of r_K on the unmodified λ chromosome.

There is a series of proteins produced by the phage which protects its DNA at various stages of infection (see refs 43 and 44). The phage coat itself is a group of proteins protecting the DNA during transfer from one host to another. The gene 2 protein of T4 protects the DNA from exo V when entering the host. We predict that analogous proteins will be found on other double-stranded linear DNA containing phage possibly gene 2 of phage T7)[44], especially since many of them also make a protein which inactivates exo V (refs 40 and 41). We also think that a protein attached to the DNA terminus may have other functions in morphogenesis, injection and expression, similar to the "pilot" protein of single-stranded DNA phage[45]. T4 phage also codes for an activity which blocks $r_{2,4}$ restriction endonuclease[42]. The presence of 20–25% of intact λ strands after infection of r_K^+ exo V$^-$ cells at a multiplicity of 2–3 may reflect unadsorbed and unrejected DNA, but it may also reflect protection of some late entering chromosome from r_K by an anti r_K-activity synthesised from the large cleavage fragments of the first genome which entered.

The restriction–modification system seems to serve as a defence and speciation mechanism which can alter the genetic effectiveness of entering DNA which it thereby defines as foreign[46-48]. Restriction cleavage of the DNA will prevent the phage production but not necessarily expression of much of its genome. Subsequently, breakdown prevents, in addition, most gene expression[29] but still makes possible rescue of many genetic loci[23,49]. We are currently investigating the expression of the cleavage products of phage DNA *in vivo*.

This research was supported by grants from National Institutes of Health and the US National Science Foundation. E.B.G. is a career development awardee of the National Institutes of Health.

Received November 14, 1975; accepted January 30, 1976.

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letters to nature

Ariel V sky survey observations of X rays from the globular cluster candidates 3U2131+11 and MXO513–40

THE high energy (2–18 keV) detector system of the Ariel V X-ray sky survey experiment (SSE) has observed the faint, high galactic latitude X-ray sources 3U2131+11 and MXO513−40 on a number of separate occasions over a period of a year (January–December 1975) and the results are reported here. These sources have previously been observed by OSO-7 (and 3U2131+11 also by Uhuru), and the globular clusters NGC 7078 and NGC 1851 respectively, were proposed as identifications[1,2]. Variability of the timescales of days to months was also observed[2].

The SSE instrument and data analysis are described elsewhere (ref. 3 and G. Villa *et al.*, unpublished). The 90% confidence error box for the source location is determined from combining the individual positional measurements by calculating the product of the probabilities of the source having been found at any given point during a single scan[4,5].

Figures 1 and 2 show the error boxes for 3U2131+11 and

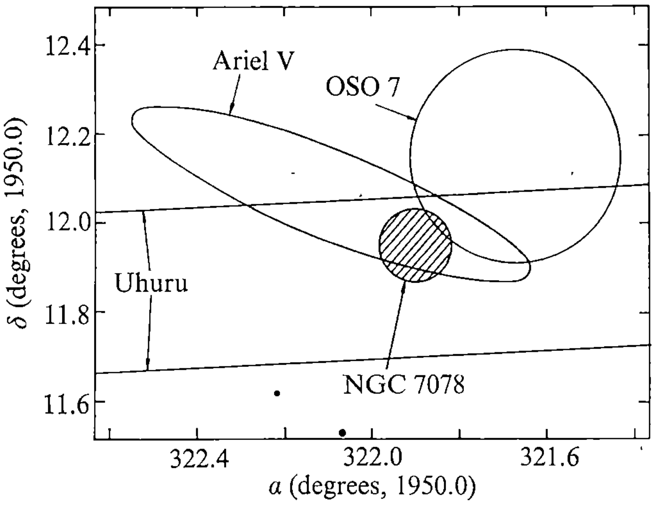

Fig. 1 Positional error boxes for the X-ray source 3U2131+11 associated with NGC 7078, from Ariel V, OSO-7 and Uhuru

MXO513−40 from the Ariel V sky survey, together with those from OSO-7 (ref. 2, and from Uhuru[1], for 3U2131+11). The Ariel V error boxes reduce the uncertainty in the locations of the sources and support the previous identifications[1,2] of 3U2131+11 = NGC 7078 and MXO513−40 = NGC 1851. The centroids of our 90% confidence error boxes have celestial coordinates (α, δ, 1950.0) and areas as follows

3U2131+11: (322.10°, 12.07°), 0.15 degree^2

MXO513−40: (78.19°, −40.13°), 0.066 degree^2

Figures 3 and 4 show, for the two X-ray sources, the intensities observed by Ariel V. Each data point consists of a summation of observations over a period of 1–2 d. Clark *et al.*[2] have reported variability of factors $\sim$2 and $\sim$5 for 3U2131+11 and MXO513−40 respectively, on timescales of days to months. For 3U2131+11 the intensities measured by Ariel V (Fig. 3) are consistent with a constant value of $\sim$2 Ariel V counts s^{-1} (2–18 keV). They are also consistent with the variability $\sim$2 reported from OSO-7 (ref. 2), but it seems unlikely, from Fig. 2, that the variability greatly exceeds this level, as all 9 statistically significant intensity measurements ($\gtrsim 3\sigma$) from Ariel V are within a factor 2 of each other.

The Ariel V observations of MXO513−40 show intensity variations of a factor $\sim$6 over periods of $\sim$10 d, consistent with the OSO-7 data[2]. The range of intensities recorded was $\sim$1–7 Ariel V count s^{-1} (1 Ariel V count $\simeq$ 2.7 Uhuru counts, 1 OSO-7 counts $\simeq$ 15 Uhuru counts[6,7] for a spectrum like that of the Crab Nebula). Of particular interest is a set of six observations centred on 17 February 1975 (MJD42460) covering a period of $\sim$20 d (see Fig. 4). These observations suggest flare-like behaviour, the source intensity increasing from < 1 to $\sim$6 Ariel V counts s^{-1} in 12 d and then falling back to the original level on a similar timescale. Combining the two 'flares' with the Ariel V data at $\sim$MJD42460 and 42605, with a similar emission peak from OSO-7 at $\sim$MJD41700 (see Fig. 4), reveals a possible periodicity in flaring of (160±20) d (or sub-multiple of this period).

Being highly evolved, globular clusters appear ideal sites for capture binary systems or massive central black holes. Both have been used in explaining the globular cluster X-ray sources, the emission being assumed to result from appreciable matter accretion (see refs 8–11). In each case the X-ray emission should arise close to the centre of the cluster, and our results are consistent with such a location. For MXO513−40, our X-ray error boxes do not favour identification with any particular optical object in NGC 1851, of those that have been suggested[12,13], though it may be significant that both the Ariel V and OSO-7 error boxes lie to the same side of the cluster centre. Therefore it is necessary to obtain a still more precise location for the X-ray source.

Finally, we note that none of the theories so far put forward explicitly accounts for the variability on timescales of days and longer, observed by Ariel V and OSO-7. Thus it is important to monitor the X-ray emission and search for correlated variability in the candidate optical counterparts. X-ray spectral data will also be useful in testing the theories for the emission[6,11].

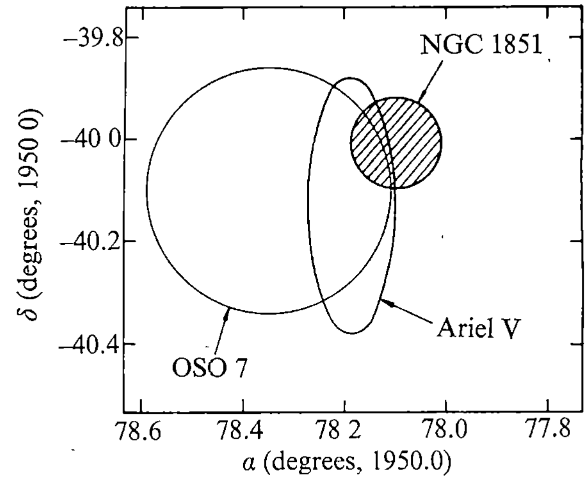

Fig. 2 Positional error boxes for the X-ray source MXO513−40 associated with NGC 1851, from Ariel V and OSO-7.

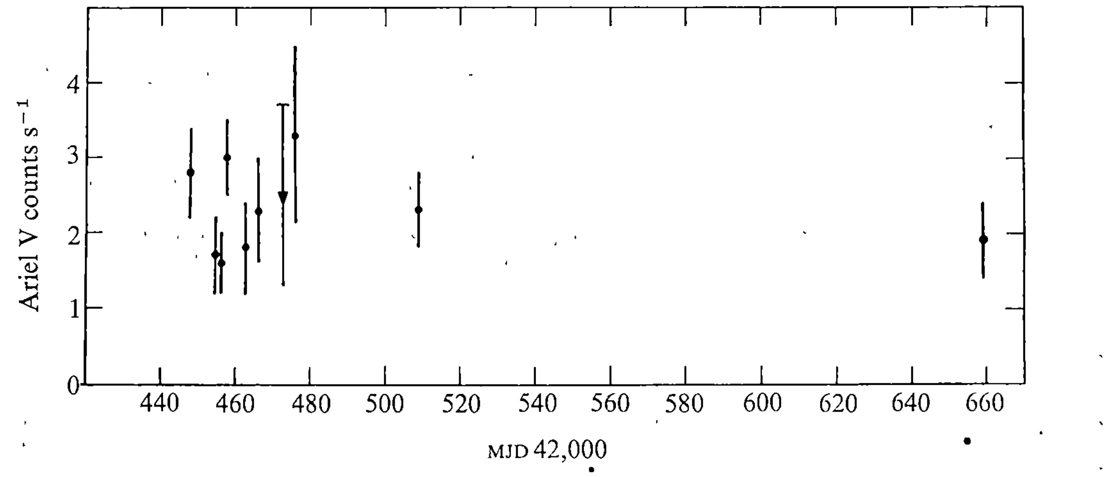

Fig. 3 Ariel V observations of intensity against time for 3U2131+11. Error bars are $\pm 1\sigma$

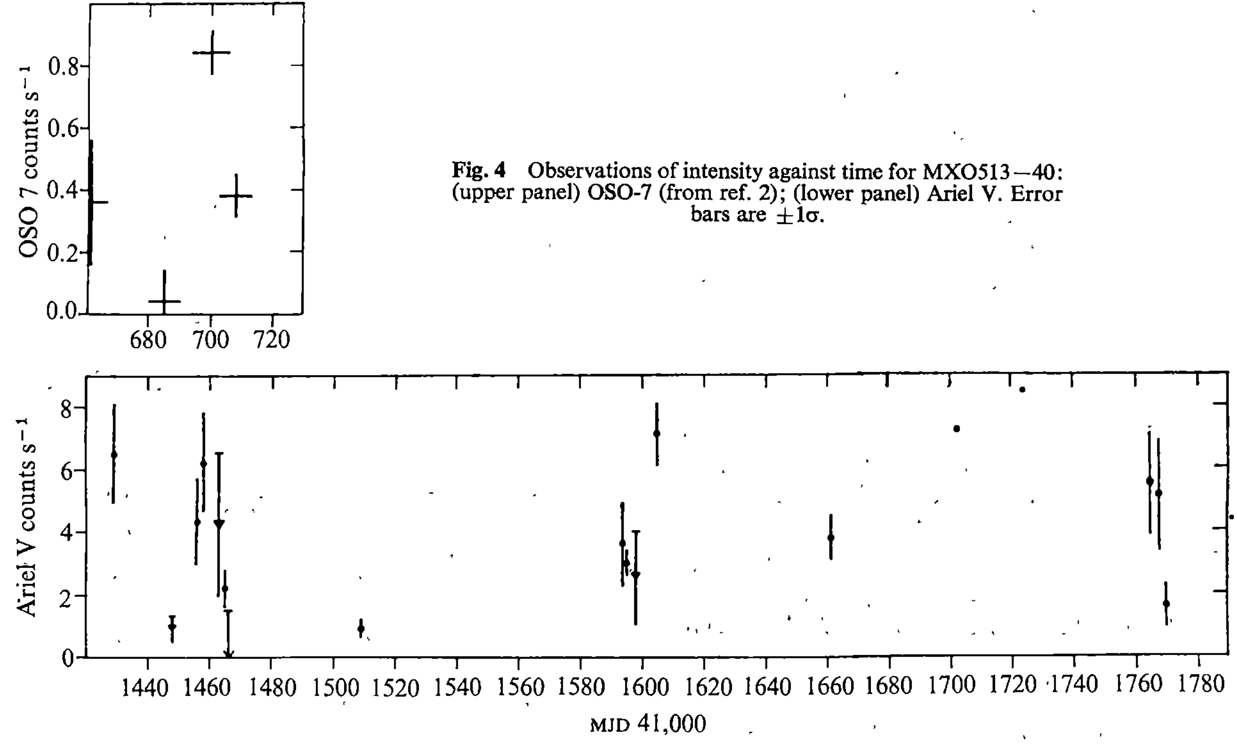

Fig. 4 Observations of intensity against time for MXO513−40: (upper panel) OSO-7 (from ref. 2); (lower panel) Ariel V. Error bars are $\pm 1\sigma$.

We gratefully acknowledge the use of data analysis computer programs written by Dr C. G. Page and M. G. Watson. The Ariel V project is supported by the SRC who also provided a research associateship for J.P.P.

Note added in proof[8]: Jones and Forman have recently reported[14] an Uhuru observation of an X-ray burst from the vicinity of MXO513−40. We note that the peak intensity of MXO513−40 observed by Ariel V could be supplied by about two such bursts per orbit.

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Received December 22, 1975; accepted February 25, 1976.

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New galactic population class

GALACTIC γ rays have been shown to come from regions where H_2 is dense, and where also 'population I' objects are predominantly found. The existence of such regions can be interpreted using the density-wave model of spiral galaxies, and leads us to propose here a change in the Baade classification scheme: 'population I' now to include all the objects associated with the H_2 clouds, and a new class, 'population 0', to refer to the regions, relatively richer in atomic hydrogen, which lie further from the galactic centre.

Satellite observations[1] imply that γ-ray emission is highly non-uniform in the Galaxy, and that its emissivity distribution peaks about halfway between the Sun and the galactic centre[2–4]. Analysis of the final SAS-2 data places this peak emissivity in the region between 5 and 6 kpc from the galactic centre[5]. The γ-ray emissivity distribution bears a strong similarity to the distribution of molecular clouds in the Galaxy[6] which also peaks in the 5–6-kpc region[7,8]. This similarity, coupled with the lack of enough gas in atomic form (H I) to explain the γ-ray measurements led to the supposition that H_2 is far more abundant in the inner Galaxy than H I, and that H_2 has the major role in producing galactic γ rays[6,8,9]. In fact a γ-ray emissivity which scales like the more uniform H I distribution cannot explain the observations. Recent studies indicate that the volume averaged density of H_2 is ~ 2–3 molecules cm^{-3} in the 5–6-kpc region and drops off dramatically at <4 kpc and in the outer Galaxy, so that at 10 kpc at least half of the interstellar gas is probably in atomic form[9]. There is a negligible amount of H_2 in the outer regions of the Galaxy[7,8]. A deduction of the implied cosmic-ray distribution from the γ-ray observations shows that the cosmic rays increase (relative to the local intensity) by a factor of ~2 (ref. 9) or slightly more[5] at a maximum coincident with the maximum in the gas density, in the 5–6-kpc region, and that the cosmic rays drop off rather rapidly in the outer Galaxy[9,10]. The cosmic-ray distribution deduced using the γ-ray observations in conjunction with the deduced variation of total gas (H I+H_2) in the Galaxy is, within experimental error, identical to the distribution of supernova remnants[11] and pulsars (refs 12 and 13, and J. Sieradakis, personal communication). The similarity of the deduced cosmic-ray distribution and the distribution of super nova remnants provides our strongest evidence to date that the observed cosmic-ray nucleons, which make up 99% of cosmic rays, originate in galactic supernovae, in either the explosion or the resulting pulsars[14].

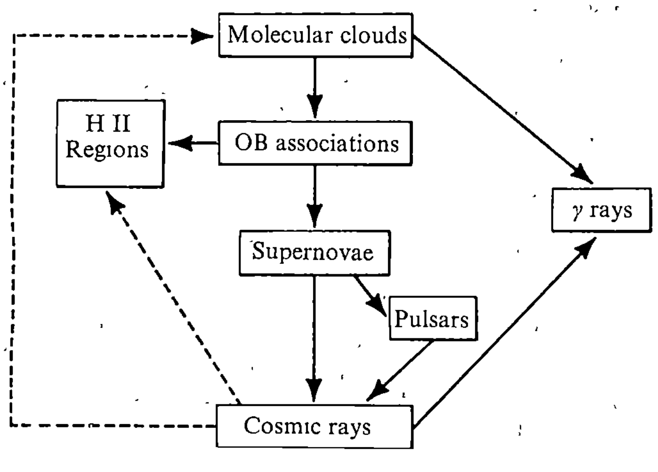
On the large scale, therefore, there appears to be an excellent correlation between several important constituents of the Galaxy in terms of their distributions as a function of galactocentric distance. These constituents are molecular clouds, H II regions (ionised hydrogen), cosmic rays, γ rays, supernova remnants and pulsars. All of these constituents of the Galaxy seem to be densest in the 5–6-kpc region and seem to drop off sharply inside of 4 kpc and in the outer Galaxy. They all can be associated with the formation and evolution of the population I stars in the Galaxy and are known to have a population I distribution[15,16]. They are associated with the formation and destruction of hot young O and B stars in the Galaxy, which delineate the arms in other spiral galaxies. That the correlation of these components is natural can best be seen in Fig. 1. The gravitational collapse of molecular clouds is expected to lead to the formation of OB associations containing the massive, hot, short lived O and B stars whose ultraviolet radiation causes the formation of zones of ionised gas around them (H II regions). The massive O and B stars, after a few million years, terminate their existence as supernovae, which in turn leads to the generation of cosmic rays. It has also been suggested that supernova explosions can trigger the formation of new OB associations 'in a feedback effect' (ref. 17, and H. B. Ogelman and S. P. Maran, unpublished). The compound effect of cosmic rays and molecular clouds being enhanced in the same region of the Galaxy then leads to an even stronger enhancement of the γ-ray emissivity in this region. In addition, an increase in the flux of subrelativistic cosmic rays may help lead to an increase in the amount of ionised gas in the region ~5 kpc, as indicated in recent surveys (ref. 18 and F. J. Lockman, personal communication).

Whereas all of the above components of the galaxy have correlated large scale galactic distributions, 21-cm radio observations of H I indicate a relatively constant overall density distribution of atomic hydrogen between 4 and 14 kpc from the galactic centre with no evidence for a significant enhancement in the 5–6-kpc region[8,19]. This implies that the H_2 distribution is much more sensitive to the compression effects expected in density-wave models of galactic structure than the more diffuse H I with the ratio H_2/H I having a radial galactic dependence somewhat similar to that of H II/H I as discussed by Shu[20].

There is a large variation in structural details among spiral galaxies. This range of detail, from those with long thin well developed arms and high surface brightness (van den Bergh type I) to those with only a bare hint of arm structure (van den Bergh type V) has been incorporated into the general framework of density wave theory by Roberts *et al.*[21]. The galaxies with well developed arms and high surface brightness with an implied star formation rate are found to satisfy the condition $(W_\perp/a) > 1$ where $W_\perp$ is the velocity component of baxic rotation normal to the spiral arms and a is the effective acoustic speed of the interstellar gas. In the inner regions of galaxies, there can exist zones of strong nonlinear compression where $(W_\perp/a) > 1$ and in the outer regions, zones of weak linear compression where $(W_\perp/a) < 1$. Burton[22] has estimated that the interface between these two zones in our Galaxy occurs at a galactocentric radius $R \sim 10$ kpc.

Figure 2 shows the smoothed radial distribution of mean surface densities of the atomic and molecular components of interstellar gas in our Galaxy based on the recent data of Burton *et al.*[8], where the H_2 density is normalised according to the methods of Stecker *et al.*[9] and using a scale height of ~65 pc for the molecular cloud distribution (based on the larger sample given in ref. 7). Also shown are the regions of weak and strong compression. It can be seen that the transition region near 10 kpc is one in which the total surface density is roughly constant but where larger and larger amounts of gas are converted from H I to H_2 as R decreases.

Fig. 1 Relationship between various 'population I' galactic components.



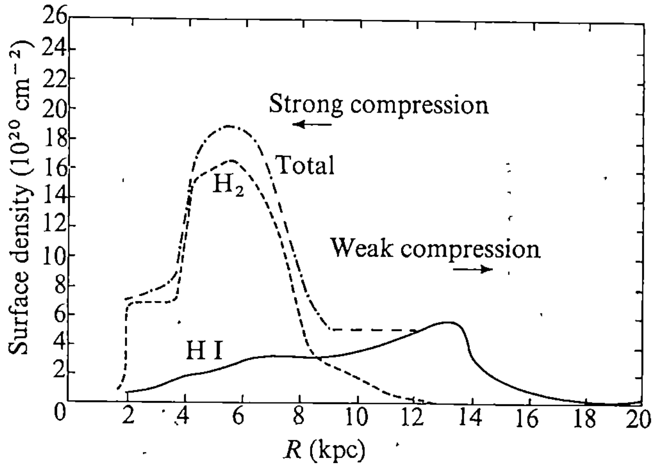


Fig. 2 Surface density distribution of H I, H_2 and total gas as a function of galactocentric radius (see refs 8, 9). The H_2 data are slightly different than those given in ref. 7, but can be considered qualitatively the same for the purpose of the present discussion. The graph illustrates the general separation of H I and H_2 components in the Galaxy and the correlation of these components with the weak compression and strong compression regions of the galactic disk respectively.

These recent observational and theoretical developments (and see also ref. 22) prompt me to suggest the following changes in the Baade[23] classification scheme for galactic objects: first, the classification 'population II', which consists of old disk stars ('high velocity stars'), nuclear bulge stars, halo stars and globular cluster stars stays the same.

Second, the classification 'population I' should be expanded to include all galactic objects narrowly confined in the galactic plane and associated with the formation of population I stars. Thus the set of galactic objects of population I will include molecular clouds, OB associations, H II regions, dark nebulae, dust, supernovae, and even associated radiation fields such as infrared, synchrotron and π^0-decay γ radiation from molecular clouds. This population is expected to predominate in regions of the galaxy where $(W_\perp/a) > 1$ (strong compression)[8,21,22].

Third, I define a new class, 'population 0', consisting of the more diffuse atomic hydrogen which is now considered not to have a primary role in star formation. (In the case of some of the denser H I clouds there may be some blurring of definition.) This population will be important in regions where $(W_\perp/a) < 1$ (weak compression). The main distinction between populations 0 and I stems from the effect of compression with the higher compression regions caused by the nonlinear density waves. These effects result in the marked differences in distribution and dynamics between the two populations. For instance, the population 0 component will have a scale height perpendicular to the galactic plane $\geq$ 110 pc, and a galactocentric radius of maximum surface density of 12–13 kpc. For population I objects these figures would be ~40–70 pc and 5–6 kpc respectively.

It is found that in late-type spiral galaxies it is characteristic for the neutral hydrogen density to peak well outside the visible radius of the galaxy[24]. The above classification, with population 0 removed from a primary role in the star formation process, naturally accommodates this hitherto somewhat mysterious fact.

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Received November 17, 1975; accepted February 11, 1976.

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Volcanic triggering of glaciation

AN instantaneous glaciation model for the formation of the large Pleistocene ice sheets has been proposed by Flohn[1] and by Ives *et al*[2]. It depends on the sudden buildup of a permanent snow cover over sub-Arctic upland plateaux including Baffin Island, Ungava, Labrador and Keewatin, Melville Peninsula, and the Finnmark Plateau and upland Sweden. The crucial event in this sudden snow buildup is the survival of snow over a large area for a single summer which then results in a series of feedback reactions leading to the establishment of permanent snowfields and, subsequently, icefields. I suggest here that such a survival could have resulted from one or several closely spaced massive volcanic ash eruptions.

Barry *et al.*[3] have stated that the climatic change needed to initiate the buildup of large permanent sub-Arctic snowfields may be quite small, but no quantitative estimates have been made of the conditions which could result in the large-scale survival of snow for a single summer. The most likely of these conditions would be a greater than normal winter snow accumulation followed by a cold, cloudy (or hazy), snowy summer, with a dearth of warm storms and a greatly decreased input of solar radiation. Volcanic eruptions over the past several centuries for which climatic data are available have enhanced these conditions, as is evidenced first, by a decrease in solar radiation which in latitudes 30–50° varies from 20 to 30% (refs 4 and 5); second, a decrease in the daylength of effective summer insolation[6]; third, a decline in mid-latitude yearly temperatures of 0.5–1.8 °C and in summer temperatures of up to 2–3 °C, (these declines lasting from 3 to 7 yr, and 10–15 yr at the higher latitudes)[5-7]; and fourth, a possible increase in precipitation (many of the coldest and wettest summers in Europe, North America and Japan have followed volcanic eruptions[6,8]) resulting from increased stratospheric dust of micron and sub-micron size which could descend into the troposphere and serve as freezing nuclei[9].

The effects of volcanism on temperature would be at least twice as great at the higher latitudes judging by temperature data supplied by Schell[10]. Volcanism would also lead to fewer warm storms over the sub-Arctic, which would mean less snow pack ablation[7,11], and to a higher frequency and intensity of summer snow storms with a resulting increase in albedo.

Such climatic effects came to maxima during the two largest volcanic eruptions since AD 1500—in 1815 and 1835. The eruptions respectively had Dust Veil Indexes of 3,000 and 4,000, total volume of tephra of 100–300 km^3 and 50–150 km^3 and volumes of atmospheric dust production of around 15 km^3 and 12 km^3 (ref 6). These eruptions were considerably less intense than some of the large Pleistocene eruptions, one of which[12] had a rhyolytic pumice and ash production of more than 900 km^3. The maximum yearly mid-latitude temperature decline in the first several years following the 1815 and 1835 eruptions was around 1.0 to 1.7 °C, and the maximum decline in summer temperatures was 2.2–2.3 °C. The temperature depression following a massive eruption of around 900 km^3 would depend on the total atmospheric ash production from the eruption and could be at least three times those averages in mid-latitudes and at least six times in sub-Arctic regions. There is also the possibility that a massive eruption north of the equatorial region at the right time of year would block almost all direct solar radiation during the entire summer in the sub-Arctic.

Considering the possible climatic effects of volcanism, I suggest that a massive tephra eruption (that is, 300–900 km^3 or larger) could have produced sufficient atmospheric dust to promote the summer survival of the snow pack on sub-Arctic plateaux. Survival would be especially likely if there were large eruptions two or more years running. Once such a survival of summer snow had occurred, it could have led to the autocatalytic growth of permanent snowfields as outlined by Flohn[1] with the following modifications: first, as a result of the original ash eruption or eruptions, the feedback process would be reinforced, leading to a greater probability of snowfield survival in the second and subsequent summers, since the lowered temperatures and decreased solar radiation would occur for at least several summers, especially at the higher latitudes; second, increased albedo and lower temperatures could result from an increase in the area of sea ice (such as occurred after the 1912 Katmai eruption[13]), which would augment the increased land albedo resulting from the survival of summer snow. Flohn[1] has indicated that the high surface albedo of a snowfield that has survived a single summer would prevent its early destruction and has outlined some steps by which a 15-m tall forest on the Ungava plateau could be covered by snow within 22 yr after a summer in which the upland snow did not melt.

If a sub-Arctic snow pack survived into early or mid-summer as a result of a massive ash eruption, then there is a possibility that the thermal condition of the land surface could contribute to further snow survival. An example[14] of this sort of feedback mechanism is the anomalous summer of 1968 during which cold sodden ground and an increased level of snow survival in parts of northern Eurasia in the early summer led to the formation of a stationary upper cold trough over north-eastern Europe and north-western Siberia. The trough persisted with the aid of thicker sea ice to the north, and it contributed to the survival of the snow pack in some areas until late June or early July and to the occurrence of early snowfalls from late July to late August. Flohn[1] has suggested that such a superimposed cold low resulting from the large-scale survival of summer snow would force the upper air to curve cyclonically and thus increase snowfall.

There is evidence of an increased area of permanent snow on the sub-Arctic plateau during the recent Little Ice Age[2,3,14,15] and during a previous neoglacial phase around 2,400 yr BP (ref. 16). It is not possible to estimate the relative importance of volcanism in those increases, though both periods were characterised by significant increase of volcanic activity[17]. Whatever the total increased area of snowfield, it was not sufficient to trigger the formation of a large ice sheet, and as a consequence both periods could be considered "abortive glaciations" in the sense of Flohn[1] and Ives *et al.*[2]. Had a massive eruption occurred during these phases of increased volcanism and glaciation, then it is quite conceivable that an increase in the area of permanent snow cover might have occurred to the point at which the albedo increased and temperature fell sufficiently to permit the snowfields to survive. If that occurred, then large, mid-latitude ice sheets may have subsequently formed.

For the volcanic triggering proposed here to operate, the Earth must be in the proper stage of its glacial–interglacial cycle, that is, a genuine interglacial must be occurring so that there is sufficient heat in the oceans to energise the transport of large volumes of water over the sub-polar plateaux[18].

If a massive ash eruption occurs when the Earth is experiencing a major ice-sheet advance, then it would have little climatic impact, nor, according to Adam's model[18], would a large eruption have much impact in the early stages of glacial retreat. If, however, a large ash eruption occurred after a massive retreat of the mid-latitude ice sheets, then sufficient cooling over upland plateaux could result in year-round snow cover. If so, then a major glacial readvance could occur, always assuming that sufficient heat had been stored in the oceans to transfer enough water to the snowfields.

The possibility that large Pleistocene ice sheets were initially triggered by massive volcanic eruptions is not contradicted by the geological record. There is an apparent correlation between major phases of global ice advance and ash eruptions in New Zealand, Japan and southern South America over the past 42,000 yr (ref. 17). A compilation of further information on volcanism from these areas and from North America and Europe, which I have recently made, supports this conclusion. The much higher rate of explosive volcanism throughout the Pleistocene has already been suggested as an indication of a possible link between volcanism and glaciation[19,20].

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Received November 11, 1975; accepted February 8, 1976.

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Changes in V_P/V_S before the Tokachi-oki earthquake of May 16, 1968 off NE Japan

THE detection of premonitory variations in the ratio of compressional to shear wave velocities is one of the most promising tools for earthquake prediction[1,2]. Changes in V_P/V_S for some moderate sized earthquakes ($M \simeq$ 5–6) located on Japanese islands have been found[3,4], and for shallow, small to moderate sized earthquakes ($M < 7$) located on continents. For large submarine earthquakes ($M \simeq 8$), however, velocity changes have not previously been reported.

The Tokachi-oki earthquake of May 16, 1968 ($M = 7.9$; focal depth 0 km) occurred near the junction of the Japan Trench and Kurile-Kamchatka Trench. Although earlier work[3] had shown no changes in seismic velocities (nor for the Sanriku-oki earthquake ($M = 7.5$) of 1960, also in the same area), I have carefully reanalysed arrival times before these two earthquakes and found significant variations in V_P/V_S. So far as I know, this is the first time that a change in V_P/V_S before and after a great submarine earthquake ($M \simeq 8$) has been detected.

The events that occurred in the focal zone of the Tokachi-oki earthquake (May 16, 1968) during 1951–74 were selected from the Seismological Bulletin of the Japan Meteorological Agency for analysis. The 1968 Tokachi-oki epicentre and after-shock area, together with the stations used for this analysis are shown in Fig. 1. The swarm earthquakes of 1952 (maximum $M = 6.5$) and the Sanriku-oki earthquake of 1960 are also included. Three station combinations, such as Hachinohe(HAC)–Aomori(AOM), Miyako(MIY)–Akita(AKI) and Urakawa(URA)–Obihiro(OBI) were selected for the V_P/V_S calculations. The apparent ratio of compressional to shear velocities was calculated by

$$V_P/V_S = [t_S(A) - t_S(B)] \,/\, [t_P(A) - t_P(B)]$$

where t_P and t_S denote arrival time of P and S waves at the corresponding stations. The V_P/V_S ratio from each combination was selected with the restrictions that the source depth must be $\leq$ 40 km, and that $1.00 < V_P/V_S \leq 2.00$.

The results are illustrated in Fig. 2. Figure 3 shows a typical example of Δt_S against Δt_P for chosen periods of time for the HAC–AOM combination. Long range premonitory changes in V_P/V_S before the 1968 Tokachi-oki earthquake are clearly seen for the HAC–AOM and URA–OBI combinations, but less so for the MIY–AKI combination. These anomalies in V_P/V_S cannot be attributed to a biased distribution of events used for the analysis because there is no obvious difference in the geographical distribution of epicentres for the normal period and the anomalous one (see Fig. 4).

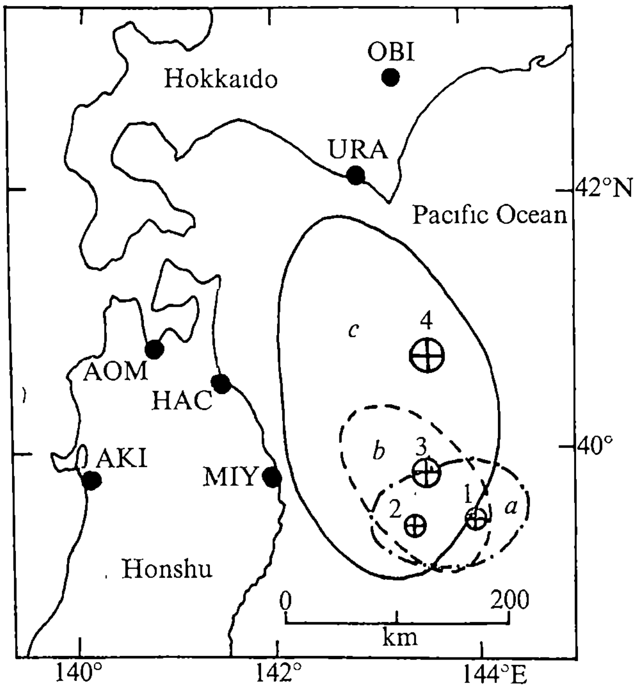


Fig. 1 Locations of the 1968 Tokachi-oki earthquake and siesmograph stations of the Japan Meterological Agency (●) Region *a*; area of the swarm earthquakes in October 1952 with the two largest events of magnitude 6.5 (refs 1, 2) Regions *b* and *c*; after-shock areas of the Sanriku-oki earthquake of March 21, 1960, with magnitude 7 5 (ref. 3), and the Tockachi-oki earthquake of May 16, 1968, with magnitude 7.9 (ref. 4), respectively.,

This long range variation was disturbed by the 1952 swarms and the 1960 Sanriku-oki earthquake. The change before the latter was especially pronounced. The 1968 earthquake occurred 4 yr after V_P/V_S returned to normal.

We may identify only the latter half of the precursory interval, the so-called recovery phase, as is seen in Fig. 2. Unfortunately, we cannot know the V_P/V_S ratios in the decreasing stage, because of the scarcity of data published by

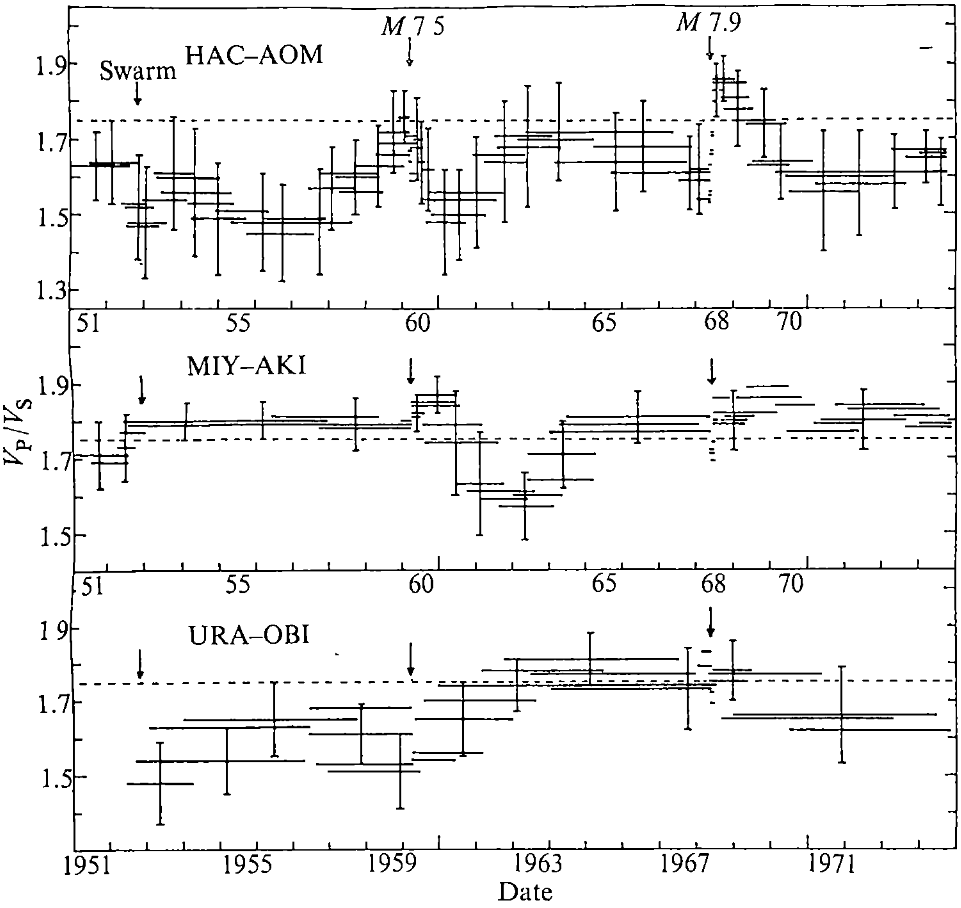


Fig. 2 The variation of V_P/V_S as a function of time before and after the Tokachi-oki earthquake of 1968, for the three station combinations. Horizontal bars show the means for a running time window of ten events stepped by two with its length indicating the time period covered by each window Vertical bars indicate the 90% confidence limits of the means of some representative windows.

the Japan Meteorological Agency before 1950. The latter half of the precursory interval before the 1968 Tokachi-oki earthquake, however, indicates that a 10–17% decrease in V_P/V_S had occurred, assuming a normal value of 1.75.

Whitcomb *et al.*[5] have proposed that the earthquake magnitude (M) and precursory anomaly time (t), deduced from V_P/V_S data, are related by

$$\log t = 0.68M - 1.31$$

where t is in days. From this, the precursory intervals are expected to be 17 yr for an $M = 7.5$ event and 32 yr for $M = 7.9$, respectively. Consequently this analysis is considered to cover almost the whole recovery period, although we cannot decide

Fig. 3 Plots of Δt_S as against Δt_P for three typical periods of time in HAC–AOM combination, each interval including ten events. *a*, Anomalously low stage, October 1, 1955– August 22, 1957, mean and 90% confidence limits V_P/V_S = 1.45±0 13. *b*, Normal stage, May 17, 1963–March 31, 1965, V_P/V_S = 1.72±0.13. *c*, Anomalously high stage, June 26, 1968–May 3, 1969; V_P/V_S = 1.85±0.06.

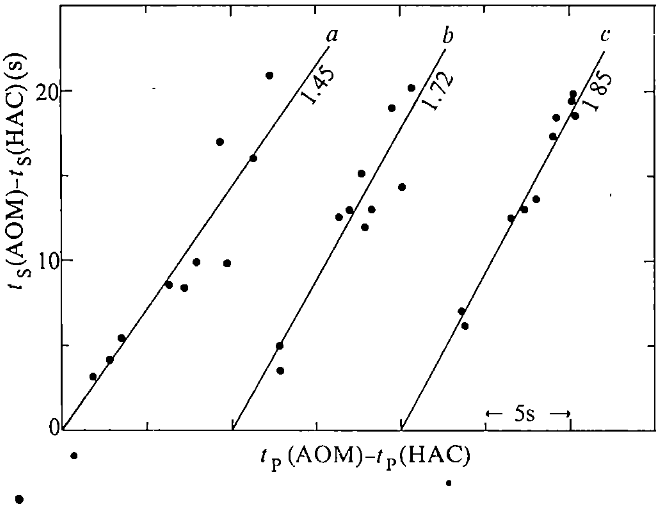
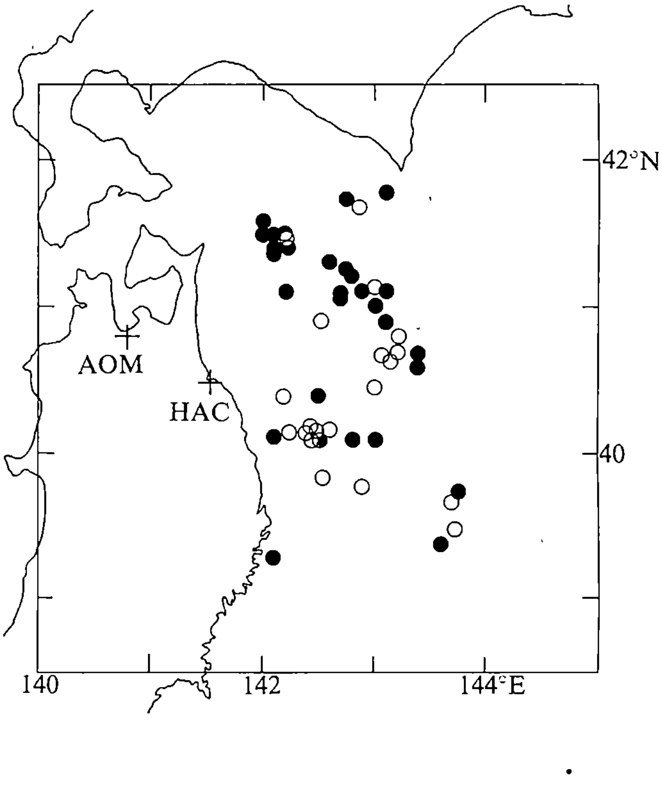


Fig. 4 Distribution of epicentres during two typical periods in HAC–AOM combination. ●, Epicentres in the anomalous stage (1953–57); ○, the normal stage (1963–67). There is no obvious difference in geographical distribution of epicentres between these two stages.



the starting time of premonitory changes in V_P/V_S ratio for lack of the data.

I thank Professor Masami Hayakawa for his encouragement during the course of this study and critical reading of the manuscript.

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Received October 23, 1975, accepted February 11, 1976

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Experimental examination of the gravitational inverse square law

IN a previous paper[1] we pointed out that earlier data on the measurement of the gravitational constant suggest a systematic shift in the value with the separation of the masses. This laboratory has been examining this question for about a year, and the early data gave a discrepancy of 0.4%. New, intensive experiments have given 92 data points, with a resulting discrepancy of 0.37%.

Our experiment compares the gravitational constant at a mass separation of 29.9 cm with the value at a separation of 4.48 cm. To expedite this comparison the gravitational torque signals are kept about the same by using a much larger mass at the greater distance. Following the ingenious suggestion of Faller and Koldweyn (unpublished; see also ref. 2), we have used rings for the attracting mass because the field is very flat at the 'Helmholtz' point and thus the positional accuracy need not be very great. The far ring is made of centrifugally cast brass and has a mass of 57,580.83 g. The near ring is made of pure tantalum with a mass of 1,225.271 g. The far ring has an outside radius of 27.112 cm, an inside radius of 21.589 cm, a thickness of 7.633 cm, and the field maximum is located on the ring axis 17.415 cm from the face of the ring. The near ring has an outside radius of 4.5536 cm, an inside radius of 2.7513 cm, a thickness of 1.7765 cm, and the field maximum is located on the ring axis 2.6088 cm from the face of the ring.

Table 1 Error contributions to Δ_{thy}

Source	Error
Torsion pendulum assembly balance	0.00007
Big ring floor tilt	0.00005
Big ring–pendulum assembly interaction	0.00036
Small ring–pendulum assembly interaction	0.00004
Big ring mass, dimensions and position	0 00031
Small ring mass, dimensions and position	0.00012
Attracted ball sphericity	0.00001
Ring density inhomogeneity	0 0001
Total error	0.00051

The gravitational force was detected with a 48.6-cm Cavendish torsion balance suspended by a 0.002-inch tungsten fibre. The attracted ball, with a mass of 50 g, was made of tantalum and hung by two fibres 87 cm below the balance rod. This reduced the gravitational torques caused by the pendulum rod and counter balance ball. The pendulum hung in a vacuum of 1×10^{-7} mmHg and the vacuum chamber wall temperature was controlled to ±0.01 °C. Substantial effort went into mounting the apparatus to reduce vibrations (diffusion pumps vibrate badly) and the pendulum hung from a vibration damper suggested by Liebes. The position of the pendulum was detected by a beam of light which was split into a two-photodiode null detector.

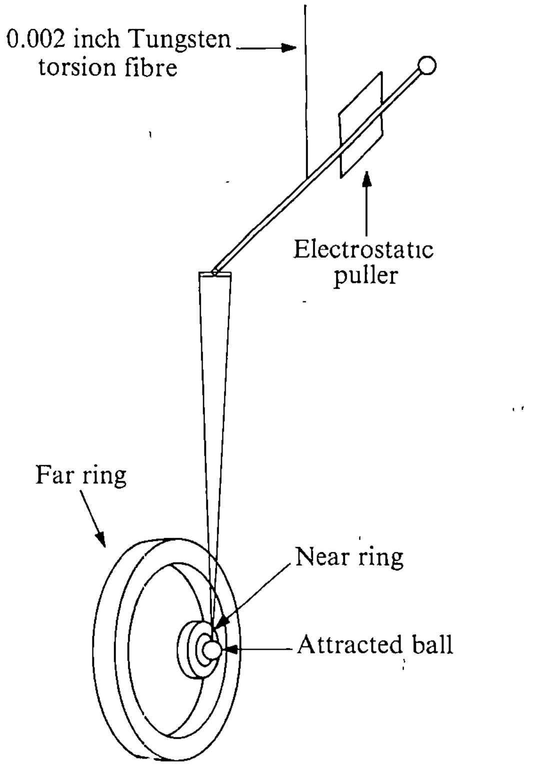


Fig. 1 Apparatus schematic illustrating the positions of the rings. The rings are used one at a time and alternately.

Another important aspect of the experiment was the use of an electrostatic puller to balance the torque of the rings. The experiment was operated in such a way that the attracted ball did not change its position appreciably. The balance voltage (squared) was the measured gravitational torque signal in the experiment; several voltages would be tried which gave an output just slightly off null and the correct null voltage would be interpolated from them.

The quantity measured in the experiment was

$$\Delta = \frac{\tau_{far} - \tau_{near}}{\tau_{near}} \tag{1}$$

where τ_{far} is the total torque produced by the far ring and τ_{near} is the total torque produced by the near ring. The Newtonian value of $\Delta(\Delta_{thy})$, was calculated by finding the torque produced by each ring on the attracted ball and each element of the pendulum assembly. Numerical integration over the rings was used to find

$$\Delta_{thy} = 0.03807 \pm 0.0005$$

where the error analysis is given in Table 1.

Table 2 Error contributions to Δ_{exp}

Standard deviation of the mean of the data	0.00015
Signal against voltage proportionality constant	0 00015
Contact potential	0.00028
Small ring torque	0.00027
Total error	0.00044

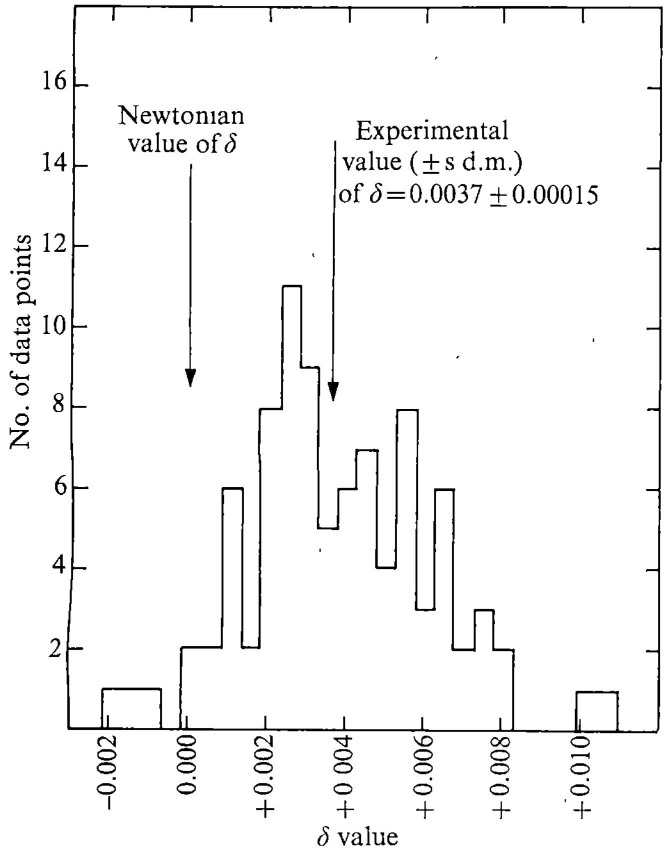


Fig. 2 Histogram of 92 δ values.

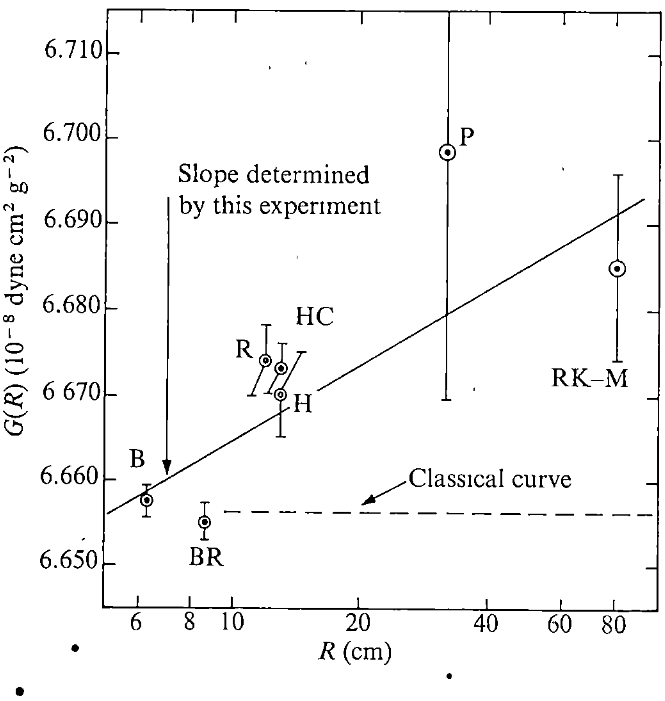
Δ_{exp} was found by directly measuring the torque difference $\tau_{far}-\tau_{near}$ by first putting one ring and then the other in position. This gave

$$\Delta_{exp} = 0.04174 \pm 0.0004$$

The discrepancy between the two results is

$$\delta = \Delta_{exp} - \Delta_{thy} = 0.0037 \pm 0.0007$$

Fig. 3 The slope of $G(R)$ given by this experiment compared with earlier data. B, ref. 3; BR, ref. 4; R, ref. 5; H, ref 6; HC, ref. 7; P, ref 8; RK–M, ref. 9.



The experimental values of δ are given in a histogram in Fig. 2.

A careful re-examination of the experiment indicates that there is no error present large enough to account for this value of δ. In particular, reversal of the rings showed, as theoretically expected, that there is no density inhomogeneity of the rings sufficient to explain this result.

The slope of $G(R)$ from this experiment is shown with G data from ref. 1 in Fig. 3. It will be seen that the two sets of data agree quite well and suggest that the gravitational force law should be modified at laboratory dimensions by

$$G(R) = G_0[1+(0.002)\ln R]$$

It should be remarked that while these results indicate an inverse square law failure at small distance, it is also clear that the experiment was carried out in the Earth's gravitational field and that we may, in fact, have a failure of the superposition principle.

I thank the many students who worked on this experiment, especially Orin Humpheries, Jim Stratton, Steve Katon, Jim Daffe, Don Ogden, and Paul Horn. I also thank John Lewis for weighing the rings, Professors R. Gibbs and E. Forsman for useful conversations, and Research Corporation for support.

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Received January 30, accepted February 25, 1976

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Organic complexes of iron and aluminium in natural waters

IN natural waters throughout the world[1], iron concentrations are commonly several orders of magnitude greater than the equilibrium solubility of iron hydroxide[2]. Highly coloured waters (which have relatively high concentrations of organic matter) commonly have even greater iron concentrations. Two of the chemical species postulated to account for this phenomenon are: (1) fine colloidal particles of iron hydroxide (possibly associated with colloidal organic matter) and (2) dissolved complexes of iron with naturally occurring organic substances. While the existence of colloidal iron hydroxide in natural waters is widely accepted[3], there is little conclusive evidence for or against the existence of dissolved organic complexes of iron. We wish to present evidence which suggests that dissolved organic matter forms complexes not only with iron but also with aluminium, with competition between Fe and Al for available complexing sites determining the relative abundances of the two metals in natural waters.

Lamar[4] has shown that substantial amounts of iron and organic matter remain in solution after filtration through 0.01-μm (100-Å) membrane filters, indicating the possible presence of dissolved organic complexes of iron. Since particle diameters < 0.01 μm have, however, been reported for colloidal iron hydroxide[5], the results of filtration studies have thus far been inconclusive.

The frequently cited correlation between iron and organic matter concentrations[4] suggests another possible means of distinguishing between dissolved and colloidal iron. A direct

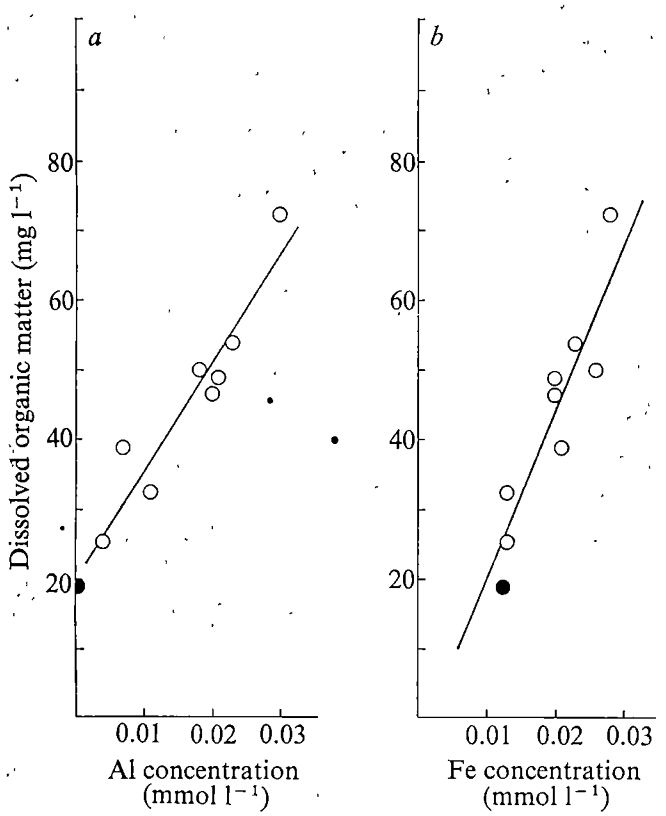

Fig. 1 Correlation between DOM (mg l^{-1}) and the concentrations of Al (*a*) and Fe (*b*) (mmol l^{-1}) for the Satilla River system (○) and for the world average river (●) The slope for Al is 1,547±222 (mg mmol^{-1}), intercept 20.1, and correlation coefficient 0.943. For Fe, these parameters are 2,378±472, −2.7, and 0.899.

correlation between iron and organic matter concentrations is more likely to result from the formation of dissolved organic complexes than from the random adsorption of colloidal iron hydroxide on the surface of colloidal organic particles. Consequently, since only a moderate correlation between iron and organic matter concentrations is usually observed in natural waters, it has generally been concluded that iron is present mainly in colloidal form. In fact, when we reported that a moderately good correlation exists between dissolved organic matter and the concentrations of iron and aluminium in the Satilla River system in the south-eastern USA, we attributed the observed scatter in the data to a lack of discrimination between dissolved and colloidal species[6].

There is, however, another reasonable explanation for the lack of a good correlation between iron and organic matter concentrations. Since it is unlikely that the organic matter in natural waters selectively complexes iron, a substantial fraction of the available complexing sites could be used to complex other metals. Natural abundances of the various metals and stability constants of the respective metal–organic matter complexes would determine the final distribution of complexed metals. For example, because of natural variations in the local relative abundances of Al and Fe in the Satilla River system, only moderate correlations are observed between dissolved organic matter (DOM) and the individual metal concentrations (Fig. 1). An excellent correlation between DOM and the sum of Al and Fe concentrations is, however, observed (Fig. 2), indicating that the total solubility of the two metals is determined by the complexing capacity of the DOM. For comparative purposes, data for the average concentrations of DOM (19.2 mg l^{-1})[7], Al (0.0004 mmol l^{-1})[8], and Fe (0.0120 mmol l^{-1})[1] in the rivers of the world are also plotted in Figs 1 and 2.

Beck *et al.*[6] have demonstrated that the bulk of dissolved organic matter in the Satilla River system closely resembles fulvic acid, an important fraction of soil organic matter which forms stable complexes with many metal ions, including Al and Fe. In low *p*H, low ionic strength waters such as the Satilla River, the stability constant for the Fe–fulvic acid complex is approximately two orders of magnitude larger than the stability constant for the Al–fulvic acid complex[9]. For Al to compete successfully with Fe for available complexing sites, Al would have to be much more abundant than Fe. In the Satilla River, analyses of exchangeable cations in the < 2 μm fraction of suspended clay minerals have shown that Al is the major metal cation and that the average Al/Fe molar ratio is > 270 (ref. 10). Thus, it is reasonable to assume that Al is present in sufficient excess to compete successfully with Fe for available complexing sites in the fulvic acid.

To calculate the molar DOM/(Al + Fe) ratio, it is necessary to estimate the molecular weight of the DOM. In a recent study, a number-average molecular weight ($\overline{Mn}$) of 1,269 was determined by vapour pressure osmometry for DOM which was isolated from the Satilla River[11]. This value is comparable with the $\overline{Mn}$ of 945 which has been reported for soil fulvic acids[9]. Assuming that the $\overline{Mn}$ of DOM in the present study falls somewhere between the experimentally determined values of 945 and 1,269, the calculated molar DOM/(Al + Fe) ratio would be between 0.8 and 1.1. Thus, metal–organic complexes of ~ 1:1 molar ratio such as those reportedly formed in metal–fulvic acid solutions[9] are also formed in the Satilla River.

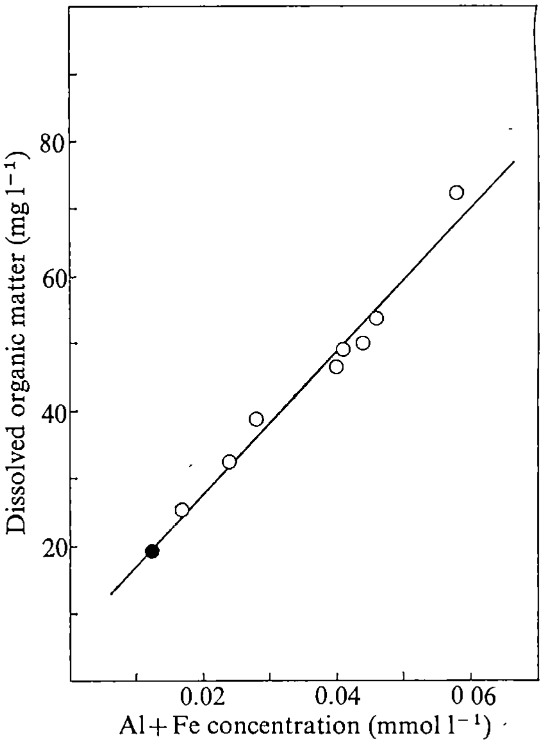

Fig. 2 Correlation between DOM (mg l^{-1}) and the sum of the concentrations of Al and Fe (mmol l^{-1}) for the Satilla River system (○) and for the world average river (●). The slope is 1,058±78 (mg mmol^{-1}), intercept 6.6, and correlation coefficient 0.984.

In Fig. 2, the calculated least squares line does not pass through the origin, possibly indicating that a small fraction of the available complexing sites are occupied by metals other than Al and Fe. Based on stability constants of metal–fulvic acid complexes[9], Ca is the only major cation which might be complexed to a significant extent. Although the Ca/(Al + Fe) ratio is ~ 1:1 in the Satilla River[6], a value of 30:1 is observed in the world average river[1]. Thus, in the world average river, a substantial fraction of the complexing sites may be occupied by Ca, leaving much of the total Al and Fe present as colloidal hydroxides. Thus, it is probably fortuitous that the ratios of DOM to Al, Fe and (Al + Fe) in the world average river are the same as those found in the Satilla River (see Figs 1 and 2). Only when actual data are available for other river systems will it be possible to determine whether the metal–organic interactions observed in the Satilla River are generally important in the transport of iron and aluminium in natural waters.

The support of the US Environmental Protection Agency is acknowledged.

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Shortening of growing season in the US corn belt

I ATTEMPT here to relate the gradual and oscillatory temperature drop in the Northern Hemisphere to a shortening of the length of the growing season in the US corn belt.

The Northern Hemisphere has cooled down during the past 30 yr (refs 1–3); Kukla and Kukla[2] have shown that the increase in the mean annual area of snow and ice over the Northern Hemisphere during the winter of 1971–72 was equivalent to one-sixth of the change necessary to bring back the full-ice conditions of the most recent ice age. Wahl and Bryson[3] reached a similar conclusion, and stated that in the past 30 yr the sea-surface temperatures measured at nine ship stations in the North Atlantic have decreased by an amount equivalent to one-sixth of the drop to minimum ice age temperatures of 17,000 yr ago. Similarly, the average length of the growing season at six climatological stations with the longest meteorological records in the US corn belt (see Fig. 1) have shortened by 14% (27 d) within the past 30 yr. Detailed study of corn belt data, however, indicates highly oscillatory year-to-year variations over the past 75–100 yr, indicating the need for caution in predicting trends.

The six climatological stations covered in Fig. 1 are located to the south and south-west of the Great Lakes. The prevailing winds are generally south-westerly during the growing season and there is great geographical variability of the observed parameters within the area. The microclimatology of some stations is affected by riverine or urban and industrial influences. For that reason I have not attempted to establish a representative average change in the growing season in the corn belt.

The growing season at Toledo (Fig. 1*a*) decreased by as much as 43 d during the last 30 yr, the largest decrease of the six stations. On the other hand, no change, or a slight increase, was observed at Peoria (Fig 1*d*), a site affected by both riverine and urban and industrial influences. Except for Peoria, the decrease in the length of the growing season at the remaining five sites varies between 22 and 39 d (Fig. 1)

The growing season is defined as the number of days between the last killing frost in the spring and the first in the autumn. It is important to realise, however, that though the length of the growing season is a significant factor, other climatic variables also impose significant limits on corn production. Thompson has stated[4] that the unusually high temperatures from 1930 to 1940, especially during the months of July and August, resulted in below average crop yields compared with those expected during normal weather. After 1940, although the length of the growing season in the corn belt was generally decreasing, both temperature and moisture were ideally distributed, and record corn yields were established. As the average growing season in the corn belt is now 165 d little further decrease can be tolerated as the minimum requirement for hybrid corn is 100–130 d. A more important factor is the ever present, large, year-to-year, oscillatory character of the growing season: for example, the abnormally early killing frosts in Iowa in 1875 (August 22), in 1950 (August 20), and in 1974 (September 5).

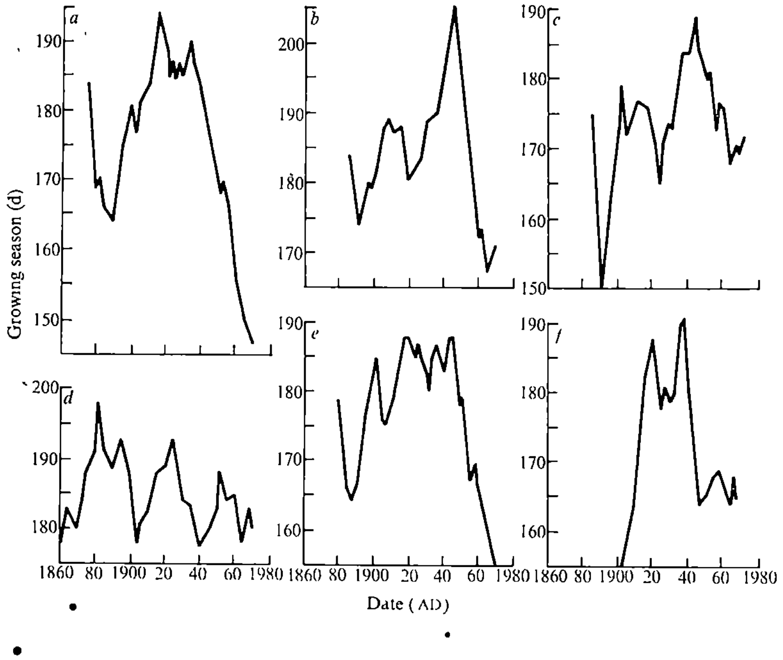

Fig. 1 11-yr running averages of the variations in the length of the growing season for six stations in the US corn belt: *a*, Toledo; *b*, Columbus; *c*, Des Moines; *d*, Peoria, *e*, Dubuque, *f*, Fort Wayne.

The variations in the length of the growing season seem to serve as a useful climatic index, directly related to the agricultural production areas and sensitively linked to the variations of the seasonal patterns.

I thank G. J. Kukla of Lamont-Doherty Geological Observatory for comments.

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Received December 19, 1975; accepted February 26, 1976.

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Entropy of vitreous ice

THE excess thermodynamic properties of vitreous solids over those of crystalline forms include contributions from two sources: first, the configurational changes that occur in the vitreous state[1]; and, second, lattice vibrations that are of a relatively low frequency and are thought to be relatively more anharmonic. We consider here the excess and the residual entropy of vitreous ice and discuss each in terms of the molecular processes and the structure.

There are at least two allotropic forms of vitreous ice[2], obtained by depositing vapours on a substrate maintained at 10 K in one case, and at 77 K in the other. The former has a density nearly 20% higher than the latter, and its oxygen pair correlation function obtained from X-ray diffraction shows an additional peak at 0.33 nm, corresponding to about 1.4 next near-neighbours[2]. It is also noteworthy that very thin deposits of amorphous films of ice on cold (< 173 K) thimbles of hygrometers[3]—a common and unwanted occurrence in meteorological experiments—may be yet another allotropic form. Only vitreous ice, obtained by depositing vapours on a substrate held at 77 K, with a 'glass transition temperature', T_g, of 135 K, is considered here.

The entropy, S, of vitreous, cubic and hexagonal ice is plotted against temperature in Fig. 1. The values were obtained from the known heat capacity of vitreous and cubic ice[4] at temperatures above 21 K, and from the calculated heat capacity below 21 K, assuming a constant Debye temperature. The entropy of hexagonal ice was obtained from the known heat capacity at temperatures above 2 K (refs 5–7). The excess entropy, S_{exc}, which is the difference between the measured values ($S_{vitreous}-S_{crystal}$), is also plotted against temperature in Fig. 1.

At high temperatures the vibrational heat capacity of solids approaches the classical limit, k, per degree of freedom. If the lattice vibrations were solely responsible for the difference between the entropies of the amorphous and crystalline solid, a plot of S_{exc} against temperature would resemble the Debye heat capacity curve; that is, it would show a rapid rise at low temperatures followed by an approach to an asymptotic value at a characteristic temperature, θ, given by, $\theta = h\nu/k$, of the frequencies, ν, that differ between the two solid forms.

The S_{exc} value of vitreous ice follows the Debye behaviour up to 85 K (Fig. 1). It shows a rapid rise in the range 5–40 K, followed by a nearly constant value of 1.2 J K^{-1} mol^{-1} in the range 55–85 K. Above 85 K, S_{exc} increases progressively with temperature and reaches a value of 2.1 J K^{-1} mol^{-1} at 135 K ($= T_g$), at which the spontaneous crystallisation to cubic ice occurs. The temperature dependence of S_{exc} in Fig. 1 is remarkably similar to that observed for vapour-deposited solid methanol, for selenium and other glasses[8] and for the disorder frozen-in state of crystals[9].

The initial increase in S_{exc} of 1.2 J K^{-1} mol^{-1} is partly attributable to the difference in the Debye frequencies and partly to the difference in the degree of anharmonicity of lattice vibrations. It is considerably smaller than the initial increase found for other amorphous solids[8,9] but seems reasonable in view of the fact that vitreous and hexagonal ice have nearly the same molar volume[2] and the same number of near neighbours at a distance of 0.276 nm.

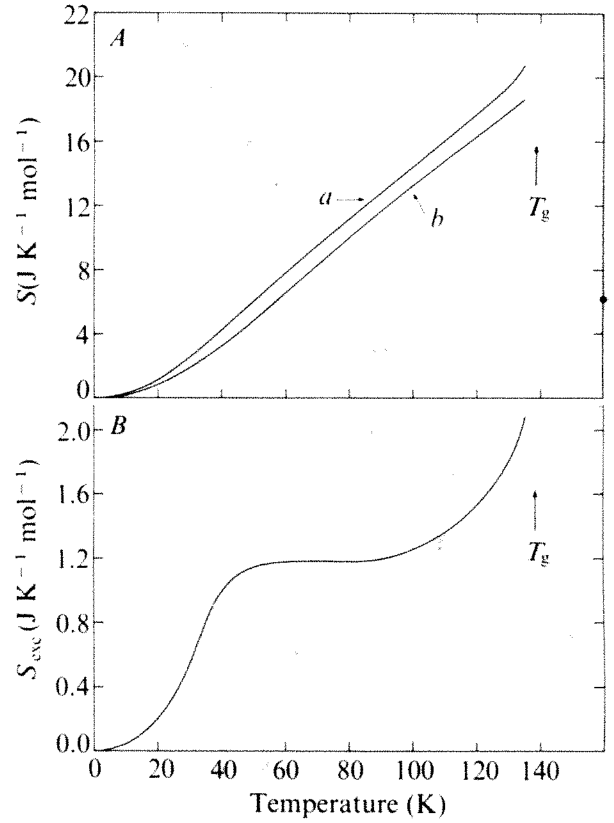

Fig. 1 *A*, Calorimetric entropy of vitreous (*a*), and cubic and hexagonal (*b*) ice plotted against temperature in the top half. Within the experimental error of 1%, the entropy of cubic and hexagonal ice are the same below 135 K. *B*, Excess entropy of vitreous ice over crystalline ice ($S_{vitreous}-S_{crystal}$) plotted against temperature. T_g, The 'glass transition temperature' at which spontaneous crystallisation to cubic ice also occurs.

The 75% increase in S_{exc} between 85 K and 135 K is no doubt attributable to the presence of configurational degrees of freedom, and indicates the onset of small-scale molecular rearrangements in vitreous ice. These may occur in some local regions surrounded by a rigid amorphous matrix, as in many molecular glasses[1], and would involve either or both of the rotational and translational motions of water molecules. These molecular motions would show up in a dielectric study as either a secondary relaxation[1] or a high value of the dielectric loss.

The residual entropy, S_{res}, of vitreous ice must, of course, have contributions from two types of disorder of water molecules; one, the positional and the other, the orientational. The contribution from the positional disorder can be readily inferred from a comparison with an analogous tetrahedrally-bonded structure. Since the continuous random network structure of vitreous ice[10] is similar to that of vitreous silica[11], the maximum value of this contribution is 5.8 J K^{-1} mol^{-1}, a value calculated for the latter from topological considerations[12]. The maximum contribution to S_{res} from orientational disorder is 3.4 J K^{-1} mol^{-1} ($= R\ln(3/2)$), as in hexagonal and cubic ice, but a realistic value would be less, for, in the vitreous state, the six orientations of a given water molecule would not be equally probable and consequently, each of the $(3/2)^N$ configurations would not have the same energy. The maximum theoretical value of S_{res}, anticipated for a fully H-bonded, fully (orientationally) disordered, vitreous ice is, therefore, 9.2 J K^{-1} mol^{-1}.

The calorimetric value of S_{res} of vitreous ice cannot be obtained from the Third Law of Thermodynamics as the course from vitreous ice to the equilibrium liquid state at 273 K involves two irreversible transformations: first, vitreous → cubic ice at 135 K, and, second, cubic → hexagonal ice in the range 160–210 K. Only the maximum value of calorimetric S_{res} can be obtained using the Clausius inequality principle. Thus: $dS > (dq/T)$, where dq is the heat evolved in the process at a temperature T. For the transformation, vitreous → cubic ice, $dq = -1.64 \text{ kJ mol}^{-1}$ at $T = 135$ K (ref. 4). Therefore, dS, which is the minimum entropy loss at the transformation, is $12.1 \text{ J K}^{-1} \text{mol}^{-1}$. The S_{res} can be written as

$$(S+S_{res})_{vitreous} - (S+S_{res})_{cubic} < 12.1 \text{ J K}^{-1} \text{mol}^{-1}$$

At 135 K, $S_{vitreous} = 20.8$, $S_{cubic} = 18.7$ and $(S_{res})_{cubic} = 3.4 \text{ J K}^{-1} \text{mol}^{-1}$. The maximum value of the calorimetric S_{res} is, therefore, $13.4 \text{ J K}^{-1} \text{mol}^{-1}$, which is consistent with the corresponding theoretical value of $9.2 \text{ J K}^{-1} \text{mol}^{-1}$.

The agreement between the maximum value of the theoretical and the calorimetric S_{res} is significant, although it does not indicate whether an increase in S_{res}, such as would occur if the vitreous ice were not completely H-bonded, is partly compensated for by a decrease in S_{res} attributable to the lack of complete orientational disorder. In the absence of the heat capacity data of liquid water between 135 and 230 K, this analysis seems to be the simplest and perhaps most appropriate.

I am indebted to Professor M. Goldstein, Yeshiva University, New York, for discussion.

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Received February 4; accepted February 20, 1976.

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Restricted pollen flow of two woodland herbs determined by neutron-activation analysis

THE movement of pollen is a critical event in the population genetics of wind-pollinated plants. Neighbourhood size and potential for genetic differentiation, for example, are significantly influenced by pollen flight distances[1,2]. The evolution of phenological and morphological mechanisms that enhance wind pollination is well studied[3,4], but the actual distance of pollen flight in natural populations is less clearly documented. Pollen movements have been estimated by using genetic markers in field plots[5], trapping pollen with artificial barriers[6], and catching radioisotope- and chemically-marked pollen of conifers[7–9]. I report the successful measurement of wind-dispersed pollen in natural populations of woodland herbs, using neutron-activation analysis of surface-treated pollen. Preliminary results suggest highly restricted pollen movement in these herbs.

Carex plantaginea Lam. and *Carex platyphylla* Carey (Cyperaceae) are common sedges in New York forests. These monoecious plants flower in early May. The florets are highly modified for wind pollination, although the different culm (stem) morphologies of the two species suggest that pollen flow might be relatively lower in *C. platyphylla*. Fertile culms elongate well before most other vernal herbs appear; thus, there are few herbaceous barriers to pollen flight.

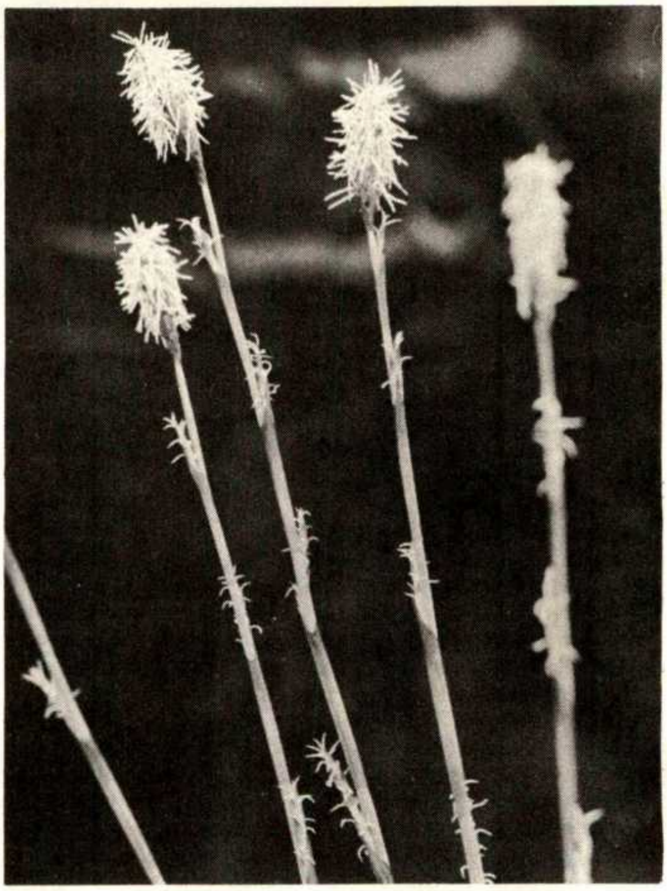

Fig. 1 Tops of fertile culms of *Carex plantaginea* during anthesis. Lateral pistillate spikelets have exserted stigmae a few days before and during opening of anthers from the terminal staminate spikelet. *Carex platyphylla* has a similar infloresceinse, but is shorter (~ 12 cm as against 20 cm for *C. plantaginea*), with lateral spikelets more clustered towards the apex, and erect foliose bracts subtending the lateral spikelets. (×0.39).

Pollen movements within a population of each species were estimated by marking pollen of selected plants with a normally absent element, samarium. After pollen dispersal, traces of samarium were searched for on stigmae of plants throughout an area uninterrupted by trees. A section of a local population of each sedge species was selected for study. These had similar densities, ~ 15 plants per m^2. Before exsertion of anthers, 15 μl (*C. platyphylla*) or 25 μl (*C. plantaginea*) of a samarium solution were applied with syringes to staminate spikelets of several central culms in each cluster. A 0.1 M solution of samarium oxide of natural isotopic abundance was prepared in hot dilute HNO_3 (ref. 10). This was buffered with 0.1 M EDTA, then the *p*H was raised to 5.7 with 1 M NaOH. For use on these species, a 0.15% solution of Tween 20 (polyoxyethylene [20] sorbitan monooleate) was added as a wetting agent.

The day after anthesis, pistillate spikelets from both inoculated plants and others in the cluster were collected into washed polyethylene vials. The wind speed was normal, $< 10 \text{ km h}^{-1}$, on days of pollen flight. Spikelets from three culms at each sample point were put in each vial, and a control series of vials was filled with *C. plantaginea* spikelets before any samarium was introduced into the population. Neutron-activation analysis of samples followed, to determine the amount and presence of samarium-labelled pollen at the various distances from the treated plants. For this analysis, the samples were bombarded with neutrons, causing unstable isotopes of samarium to be formed. The major activity comes from 47-h ^{153}Sm which β decays to ^{153}Eu, which in turn emits γ rays. The dominant emissions, 70 keV and 104 keV γ rays[11], were counted on a NaI scintillation detector. Data are summarised in Table 1.

These data record a rapid decrease of activity within short distances from samarium-treated spikelets. Ten readings from pistillate spikelets from the samarium-treated culms of *C. platyphylla* show great activity. Within 0.15 m from the inoculated culms, there is a greater than 30-fold decrease of activity. By 0.5 m from the treated spikelets, activity at some locations was halved again, and many locations recorded no activity above control levels. Beyond 1.0 m, only four readings showed activity above the control. No bias in directionality of pollen flow was seen in these data. (No samples from the central, inoculated *C. plantaginea* culms were available, as these particular culms failed to develop mature pistillate florets, a common occurrence in population of this species[13].)

The generally lower dispersal range of *C. platyphylla* pollen supports the hypothesis that the culm morphology of this species lowers pollen flow. This may be attributed in part to the lower height of the staminate spikelets on these culms. Dispersal distance varies inversely with height of pollen source[14]. Also, the subtending foliose bracts beneath *C. platyphylla* pistillate spikelets act as mechanical barriers to pollen.

Additional studies of pollen movement in these species might include flow rate throughout clusters of similar density, but containing tree trunks. Such clusters would be expected to have lower dispersal levels, as tree stands decrease pollen movement[15,16]. Also, information on congruence of movements of treated and untreated pollen, flow during high wind speeds, and correlation between pollen movement and actual gene flow are needed. Even if infrequent, long distance pollen flow could be important to genetic processes.

The wind dispersal pattern in this study is consistent with the leptokurtic pattern seen in studies using other methods. Similarly, highly restricted pollen flow is reported from many insect-pollinated populations[2]. The neutron-activation analysis results support evidence that herb populations may be quite isolated genetically from adjacent populations[17], and that local population differentiation over the range of a species may be frequently achieved[18–21]. The low pollen dispersal pattern of these *Carex* species superficially adapted for 'wide' pollen flow, highlights the need for care in postulating large neighbourhood size in their populations.

Table 1 Relative amount of samarium-treated pollen at distances from treated staminate spikelets

Distance (m)	*C. platyphylla* *A**	*C. platyphylla* *B*†	*C. plantaginea* *A*	*C. plantaginea* *B*	*Activity of B* (Mean	s.d.)
0.00	10	10			1,498	504
0.15	4	4	2	2	44	20
0.20	12	0	6	2	56	16
0.30			2	2	27	4
0.40	8	0				
0.50	8	0	6	2	22	1
0.60	4	1			36	
0.70	2	0				
0.75			2	0		
0.90	2	0	2	0		
1.00			6	3	32	11
1.10	2	0				
1.25			2	0		
1.35	2	0				
1.50			2	0		
2.50			2	0		
3.00			2	0		
3.20			4	2	19	5
3.75			2	2	23	7
5.00			4	0		

**A*, Total number of peaks counted.

†*B*, Number of peaks with activity > 1 s.d. above mean of the control series.

Samples were irradiated at the Cornell TRIGA MARK II reactor for 1 h with a neutron flux in the range of 7×10^{11} neutrons cm^{-2} s^{-1}. After 18-h decay, γ-spectrum analysis was done with a 3″ × 3″ NaI (Tl) well-counter (Baird-Atomic Model 810C) and a 256-channel pulse-height analyser (Technical Measurement Corp. Model CN-110). Scintillations were counted for 120–600 s. Counts were calculated from 40 channel widths, relativised for count time, and corrected for decay and background radiation[12].

The use of neutron-activation techniques for studies on genetic structure and processes in populations is recommended for its relative ease and accuracy[22,23]. This technique is also applicable to other ecological studies by field biologists, such as recording visitation patterns of nocturnal pollinators, feeding behaviour of nectar feeders, or occurrence of food exchanges between members of animal colonies.

I thank H. C. Aderhold and the Ward Laboratory of Nuclear Engineering, Cornell University for help in this study.

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Received December 24, 1975; accepted February 4, 1976.

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Inhibition of float formation in water hyacinth by gibberellic acid

THE water hyacinth (*Eichhornia crassipes*) is one of the most harmful weeds of tropical and subtropical waterways and lakes. Its reproductive potential is enormous and in favourable conditions it forms dense mats which may cover the surface of a body of water. Such prolific development can obstruct the drainage of farmlands, block hydroelectric installations in dams, hinder navigation and affect fisheries (reviewed in ref. 1). The leaves of free-floating water hyacinths are characterised by a bulbous swelling in a section of the petiole which provides buoyancy. These floats, however, do not develop when the plants are rooted in the soil of muddy shores. In those circumstances the petiole narrows gradually from its base to the leaf blade. We have found that the formation of floats can be inhibited by extremely low concentrations of gibberellic acid (GA_3) in the water. As a consequence of treatment the plants become unstable and partially sink below the surface of the water.

Water hyacinth plants were cultured *in vitro* in polyethylene trays (45 × 30 × 8 cm) on 1/5 strength Long Ashton medium composed of the following (mg per l of nutrient solution):

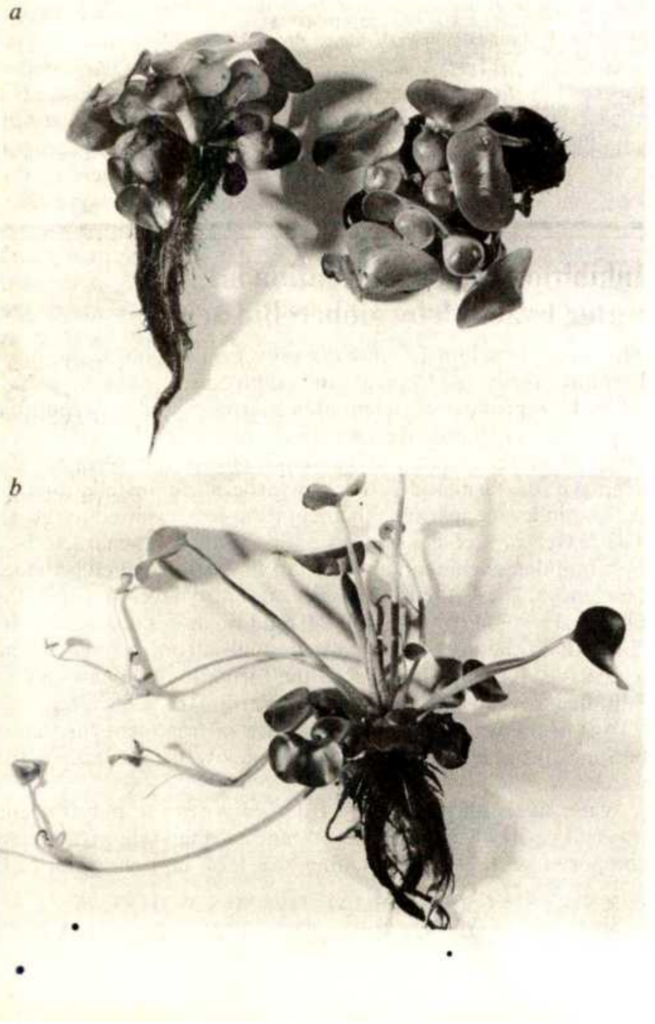
KNO_3, 81; $Ca(NO_3)_2.4H_2O$, 189; $MgSO_4.7H_2O$, 74; $NaH_2PO_4.H_2O$, 0.35; $MnSO_4.H_2O$, 0.35; $CuSO_4.5H_2O$, 0.05;

$ZnSO_4.7H_2O$, 0.06; H_3BO_3, 0.6; NaCl, 1.2;
$(NH_4)_6Mo_7O_{24}.4H_2O$, 0.02; $FeSO_4.7H_2O$, 5.6; EDTA, 5.8

adjusted to *p*H 7 with 1 N NaOH. The plants were exposed to continuous illumination (12,000 erg cm^{-2} s^{-1}) from white fluorescent tubes at 27±2 °C. Experiments were conducted in a greenhouse during the summer in reservoirs (100×100×60 cm) containing 20 cm of pot soil and filled up with tap water. The circumference of the floats and the length of the petioles were measured with a sliding gauge with Vernier scale (accuracy 0.1 mm). All experiments lasted 4 weeks. When the plants were cultured *in vitro* typical float leaves were formed (Fig. 1*a*). Increasing concentrations of GA_3, however, decreased the diameter of floats of newly formed leaves. As little as 0.03 p.p.m. of GA_3 (0.03 mg l^{-1}) in the nutrient medium suppressed the formation of float leaves (Fig. 1*b*). Even with 0.01 p.p.m. of GA_3 the floats were clearly smaller than those of controls. In Fig. 2 the quotient of petiole length and greatest circumference (*l*/*c*) is presented at different concentrations of GA_3. These values decrease markedly at concentrations of 0–0.03 p.p.m. At the highest concentration tested, 1.0 p.p.m., the value was very high because of the considerable increase in the length of the petiole. GA_3 also markedly inhibited vegetative multiplication of the plants and induced profuse flowering. 6-Benzyladenine (BA) counteracted the effect of GA_3. In the presence of BA the floats became slightly thicker than in the controls and plants with elongated leaves which had been cultivated on GA_3-containing medium reproduced floats quicker in the presence than in the absence of BA.

2-Chloroethyltrimethylammonium chloride (CCC), indol-3-yl-butyric acid (IBA), abscisic acid and the ethylene-releasing compound ethrel had no effect on float formation. Plants grown in the greenhouse in the presence of 0.03 p.p.m. of GA_3 produced only elongated leaves. These plants produced just a few daughter plants and eventually became submerged. The control reservoirs, on the other hand, were completely overgrown within 4 weeks. Preliminary spray tests in the greenhouse have shown promising results in the inhibition of float formation by GA_3.

Fig. 1 *a*, Water hyacinths after cultivation on 1/5 strength Long Ashton medium. Note the typical float leaves. *b*, As (*a*) but supplemented with 0.03 p.p.m. GA_3. Note the absence of floats in the newly formed leaves.



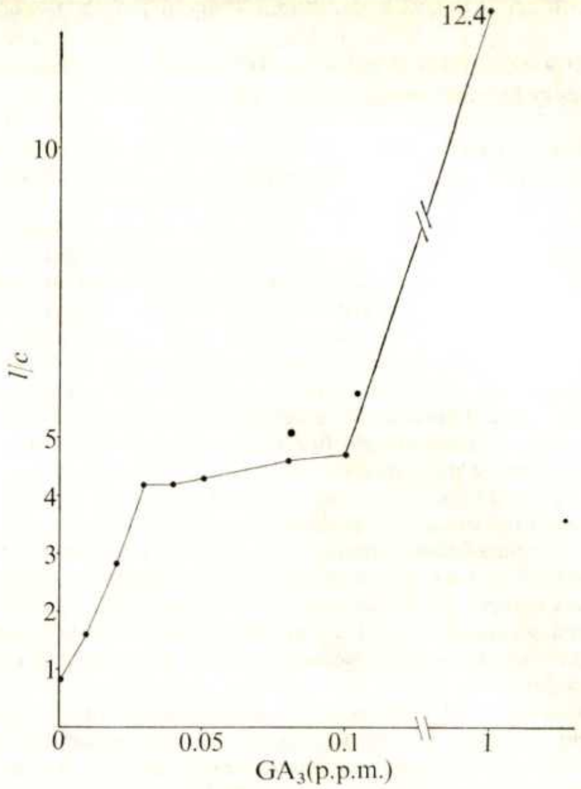


Fig. 2 Effect of various concentrations of GA_3 on float formation in water hyacinth, expressed as the quotient of length (*l*) and circumference at the greatest width (*c*) of the petiole.

In the wild, water hyacinths do not produce float leaves in crowded conditions or when rooted in the mud[2]. It has also been reported that a relatively high temperature and low light intensity (in the shade) promote elongation of the petiole[3]. Our results suggest that the external factors which influence float formation in water hyacinth act through endogenous gibberellins and perhaps cytokinins. The effective concentration of GA_3 (0.03 p.p.m.) was extremely low and as this hormone markedly inhibits vegetative growth it could perhaps be used to control the growth of water hyacinths in certain areas. It might be combined with biological means of control, such as *Neochetina eichhorniae* (water hyacinth beetle) or *Orthogalumna terebrantis* (water hyacinth mite). The very low concentrations of GA_3 that were effective would be economically feasible, and as GA_3 is a natural component of plants it should not endanger the environment.

We thank Miss Ingrid Kaczmarek for assistance.

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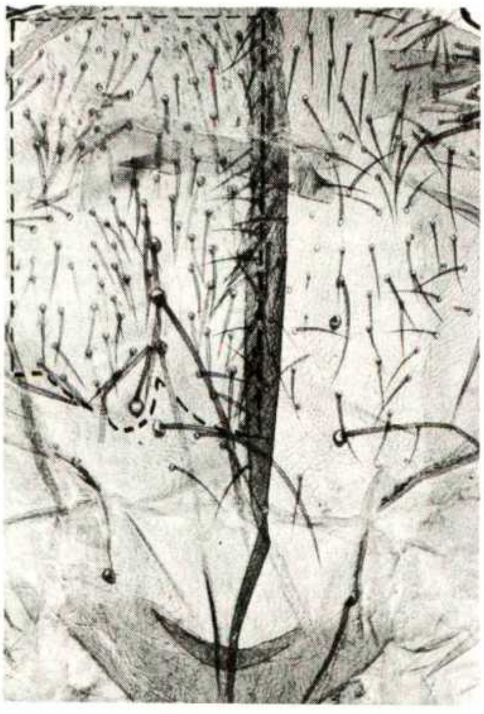
Morphogenetic mutants detected in mitotic recombination clones

GENETIC analysis has been used successfully for identifying the elements responsible for biological processes[1,2], and understanding the logic of their interactions. This approach has, however, been little used in the study of morphogenesis in multicellular organisms, chiefly because of a dearth of viable mutants and the difficulties of interpreting the altered primary gene function from the mutant phenotype. The existence of pleiotropy in particular makes this analysis difficult[3,4]. An alternative approach would be to study the effect of mutations on individual cell behaviour[5]. This approach depends on the well founded assumption that normal morphogenesis results from the ordered integration of discrete cell autonomous functions. This can be done in *Drosophila* in genetic mosaics, where the heterozygous, phenotypically wild-type individual is a carrier of clones of homozygous mutant cells. This approach has the advantage that morphogenetic mutants, even if they are lethal to the individual, can be detected, studied and interpreted directly at the cellular level.

Previous analysis in *Drosophila* has shown that about 90% of loci can mutate to a lethal condition[6,7]. Some 90% of these lethals are, however, viable in clones of epidermal cells and therefore amenable to clonal analysis[8]. Once the normal clonal parameters of a given developmental system are known[9–11], it is possible to detect mutants altering them. Here we describe a method of directly detecting and characterising morphogenetic mutants in clones produced by mitotic recombination. A similar approach has been used to detect cuticular differentiation mutants[12].

Morphogenetic mutants, induced in a chromosome arm carrying cell marker mutants, can be detected after mitotic recombination as morphological abnormalities associated with the cell marker phenotype. If the mitotic recombination event removes at the same time the *Minute* (*M*) allele in heterozygous M/M^+ growing cells, the resulting clones will be large[13] enough to be seen under the dissecting microscope. This procedure enables us to uncover mutants (1) in the chromosome arm of the *Minute*, (2) in heterozygous condition, (3) irrespective of whether they are lethal or present in a lethal-carrying chromosome and (4) as autonomous as well as partially nonautonomous cell mutations.

Fig. 1 *mwh jv* * $M(3)i^{55+}$ notum showing intercalary addition of chaetal elements.



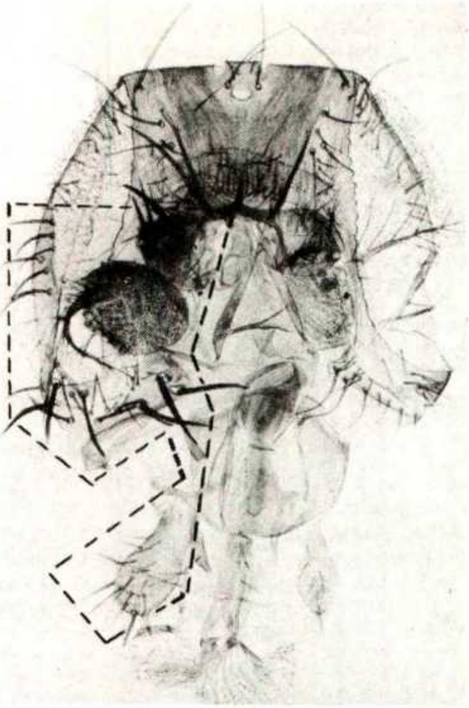
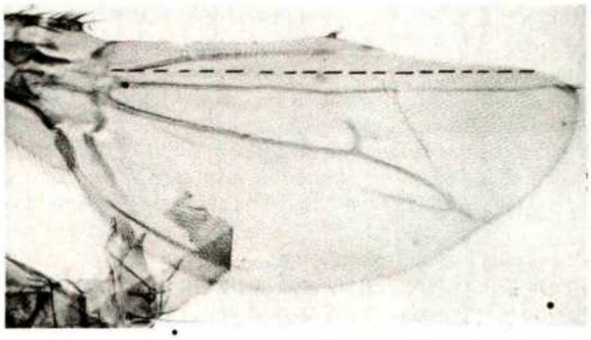


Fig. 2 *mwh jv* * $M(3)i^{55+}$ head showing cuticular elements about twice as large as wild type.

Thus, male gametes of the genotype *mwh jv*, mutations which mark the trichomes and chaetes respectively[14], were treated with ethylmethane sulphonate (EMS) (0.02 M)[15]. The treated males were crossed to balancer-carrying females (*TM1,sbd*/*TM3,e*^{11}) and the F_1 *mwh jv**/*TM1* and *mwh jv**/*TM3* females mass-crossed to $M(3)i^{55}$ *sbd*/*TM1* or to $M(3)i^{55}$ e^{11}/*TM3* males respectively. The F_2 progeny was irradiated with 500-r. X rays at 48–72 h after oviposition[16]. The emerged *mwh jv**/$M(3)i^{55}$ males were then scored under the dissecting microscope for morphological abnormalities, which could be anywhere in the body cuticle. Those males showing an abnormality were then crossed to balancer-carrying females, to ensure amplification of the treated chromosome, and then mounted on a slide and checked for the existence of *mwh jv* M^+ territories associated with the morphological abnormality. In positive cases the existence of a morphogenetic mutant was again verified in mitotic recombination clones in subsequent generations, mapped meiotically, separated from associated lethals by recombination, and maintained in balanced stocks for detailed study of its phenotype.

Out of 20 EMS-independent treatments, about 10,000 males of *mwh jv**/$M(3)i^{55}$ genotype were scored under the dissecting

Fig. 3 *mwh jv* * $M(3)i^{55+}$ wing lacking most of the anterior region. Some *mwh* cells remain.



microscope. Morphological abnormalities were detected in 95 individuals but progeny were obtained only from 68 of them. In 25 of the 68 individuals the malformation was associated with the *mwh jv* M^+ genotype, but in only 19 cases did the original malformation appear in subsequent generations. The association of the malformation with the clone of cell marker mutants indicated that it corresponds to a true mutation in the left arm of the third chromosome. Through further analysis we succeeded in assigning to each malformation a locus in that chromosome arm. All the isolated mutants have been shown to be lethal or closely linked to a lethal mutation. We tested the 19 mutants isolated for allelomorphism and found two pairs which did not complement. One of these pairs occurred in flies subjected to the same EMS treatment and can be attributed to the same mutational event and another pair corresponded to true alleles.

The different clonal phenotypes of these 18 mutants were classified as follows. A first group of five mutants lack entire regions of the adult cuticle, the surrounding territories being of more or less normal morphology (Fig. 3). They differ depending on the degree of autonomy of the lethal effect, extension of remnant *mwh jv* cells still present at the borders of the clone, and on the effect on the surrounding wild-type cells.

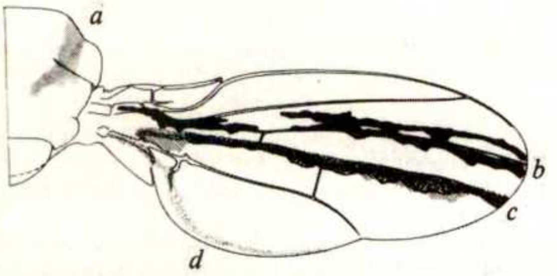


Fig. 4 *mwh* * $M(3)i^{55+}$ Diagram representing four elongated and split clones; *a*, *b* and *c* correspond to one mutant and *d* to another. Clone *c* runs along the antero-posterior compartment border in both dorsal and ventral surfaces of the wing.

A second group of nine mutants modifies the pattern of the clone in different ways. In two different alleles of the same locus the adult cuticular elements (trichomes and chaetae) are about twice as large as for the wild type (Fig. 2). Another mutant leads to the intercalary addition (2–3 times) of chaetae in an otherwise normally patterned tissue (Fig. 1). Two mutants lead to the formation of elongated and split clones, which are smaller than control ones of the same age (Fig. 4). Two mutants lead to the formation of outgrowths in the cuticular surface, one associated with whirls in the orientation of the trichomes and chaetae, and the other with thick and dark, poorly differentiated cuticle. A possible homoeotic mutant shows a transformation of the third joint of the antenna into tissue typical of the proboscis; clones in other regions of the body are of abnormal appearance.

A third group of five mutants shows different types of abnormality in the cuticle associated with chaetae and trichome pigmentation and/or morphology. They can be considered as mutations that affect the mechanisms of cuticular formation[12].

The mutant phenotypes described are suggestive of as many cell functions as can be discriminated by mutation. They possibly result from alterations in the mechanisms of growth and response to metamorphosis, cuticular differentiation, and in the control of sequential mitotic orientations, of the size of cuticular elements or of their distribution in patterns, and so on. From analysis of these and other mutants detected in this way we hope to infer the wild-type functions relevant to the genetic control of morphogenesis. These phenotypes examined at the cellular level, will also help to suggest molecular mechanisms underlying such cellular abnormalities. The efficiency of the reported method of detection of morphogenetic mutants is very high: about 1 such mutation for 500 flies scored. Using cell marker mutants[12] and *Minutes*[16] in other chromosome arms it will soon be possible to extend these studies to mutants over the entire *Drosophila* genome.

This work was supported by the Commision Permanente del Descuento Complementario (INP) and J. March Foundation.

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Received December 8, 1975; accepted January 22, 1976.

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Stereotyped pattern of locomotion controlled by duration of previous tracking by a predatory insect

AMONG insects, an encounter with a particular stimulus[1–4] or the performance of a particular activity[5–7] may change the animal's later responsiveness to other stimuli or may prime the regular appearance of a stereotyped action pattern. Thus, various unrelated species display strictly predictable behavioural changes which depend not only on simultaneous events in the external environment as well as on general physiological variables, but also on well determined sensory or motor components of the subject's

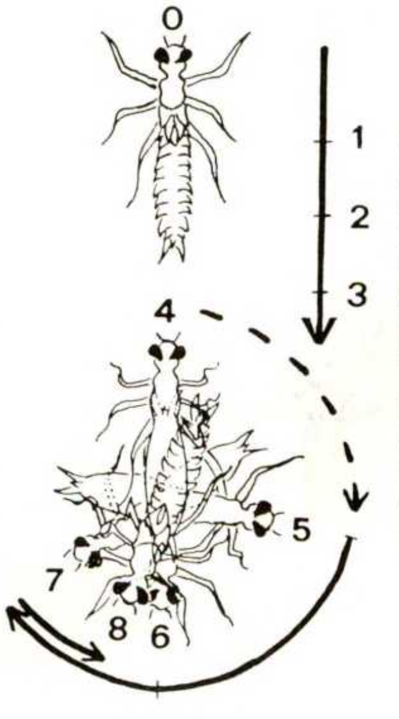

Fig. 1 The backing–turning pattern. After the disappearance of the prey stimulus, the larva (1) remains motionless (position 0); (2) it steps backwards repeatedly (solid arrow, positions 1–4); and finally (3) it turns repeatedly around its longitudinal body axis. The first pivoting movement is often accompanied by simultaneous backing (dashed arrow, position 5); thereafter, the insect turns clockwise and/or counterclockwise without changing its location (solid arrows, positions 6–8).

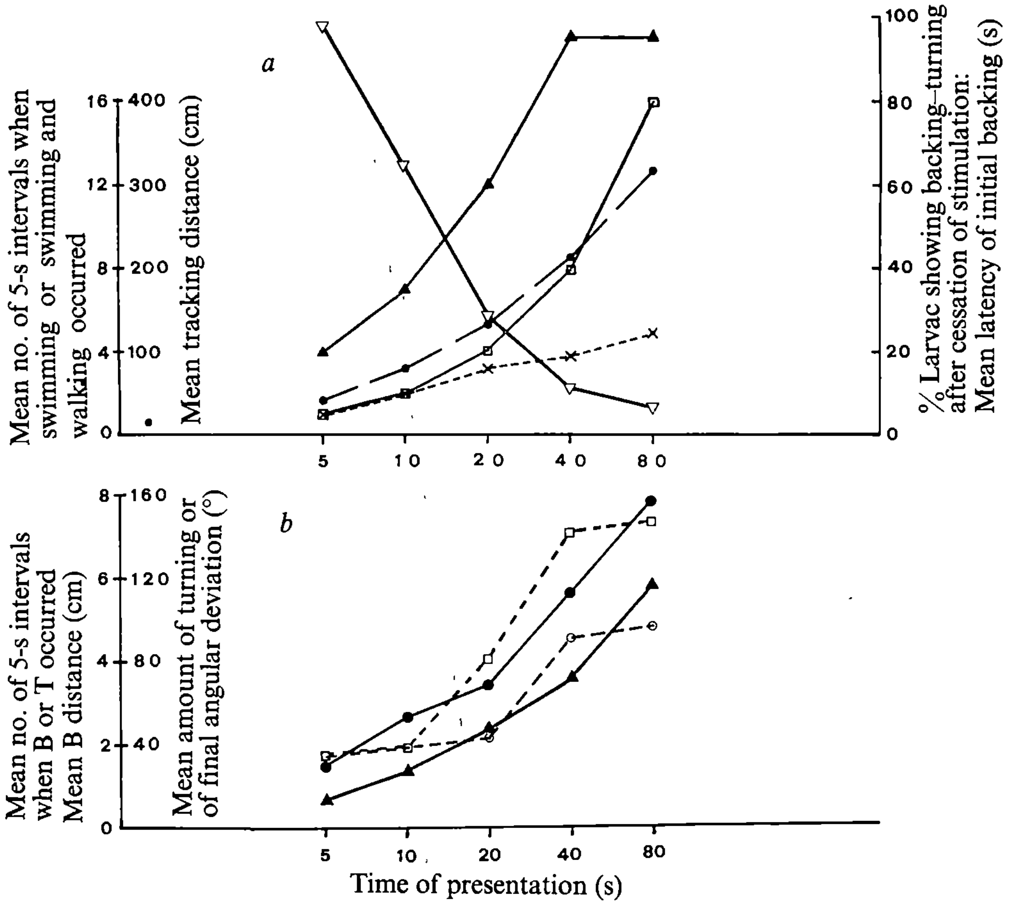

Fig. 2 *a*, Tracking time and tracking distance during presentation of the dummy for various times; percentage of larvae showing the backing–turning response within 3 min after removal of the dummy; and latency of initial backing response. B, Backing; T, turning; S, stimulation; ×, swimming time; ●, tracking distance; □, total tracking time corresponding to swimming and walking; ▲, backing–turning after stimulation; ▽, latency of initial backing. The increase in the number of individuals showing the backing–turning response and the decrease in the latency of the initial backing response are significant at the 0.01 and 0.001 levels, respectively. The relationship between the time of presentation and the percentage of individuals showing backing–turning corresponds to the equation $B\text{–}T = 0.517t^{1.564}$ (where t stands for time of presentation) $n = 100$ *b*, Number of backing–turning responses, backing distance and amount of angular turning within 3 min after removal of the dummy. ●, Backing–turning response; □, turning; ▲, backing distance; ○, final angular deviation corresponding to the change in the orientation of the insect's head and body resulting from the entire sequence of backing–turning movements. Except for the final angular deviation, the changes in the values of the different backing–turning parameters are all significant at the 0.001 level $n = 60$

antecedent behaviour. The study of these changes may aid the understanding of the nature of specific internal mechanisms which are influenced by previous behavioural states and which act in turn on the animal's subsequent behaviour. This may be of special interest in the study of highly elaborate action patterns, such as the figure-of-eight dance of the honey bee. In this example, the return of the bee from a given food source to the hive is followed by a precisely defined pattern of locomotion, in which the timing and orientation of certain parameters depend on specific aspects of the insect's outgoing flight[8]. A series of experiments analysed the reactions of the aquatic dragonfly larva *Aeschna cyanea* M. after a brief presentation of an artificial prey stimulus They revealed that the behaviour of the insect is influenced by the nature and former location of the stimulus, even after the latter has disappeared[9]. On the other hand, after a more extended presentation of a moving target—a stimulus which typically elicits prolonged tracking behaviour—the insect reliably carries out a strongly stereotyped pattern of locomotion, the form, control and function of which are described here.

Hungry subjects of the final larval stage were placed in a large experimental aquarium (70×70 cm) and presented with a spherical dummy prey (diameter, 3.5 mm) for 5–80 s. The dummy was introduced directly into the insect's visual field and then moved around the aquarium, its velocity being adapted to the subject's own tracking speed. At the end of the period of stimulation the dummy was removed quickly from the aquarium. The behaviour of the larvae during the presentation of the dummy as well as during the 3 preceding and 3 subsequent minutes was recorded by two cameras, one of them stationary, above the centre of the aquarium, the other fitted with a zoom lens and manipulated by the experimenter in front of the aquarium.

Presentations of the dummy during 5- and 10-s intervals elicited tracking with vigorous swimming movements; presentations of still longer duration elicited swimming movements followed by rapidly decelerating walking (and creeping) movements (Fig. 2*a*). After removal of the dummy, the insects tended to display a stereotyped pattern[10] which had not been observed before or during the presentation of the dummy. It consisted of three phases: (1) the larvae remained motionless and 'stared' in the direction in which the prey stimulus had just disappeared; then they carried out (2) a series of backing movements and finally (3) a number of turning or pivoting movements around the longitudinal axis of their body, without changing their location (Fig. 1).

The longer the preceding presentation of the dummy, the sooner the 'backing-turning' pattern appeared, and the greater (1) the percentage of larvae which showed this pattern (Fig. 2*a*), (2) the number of time units in which

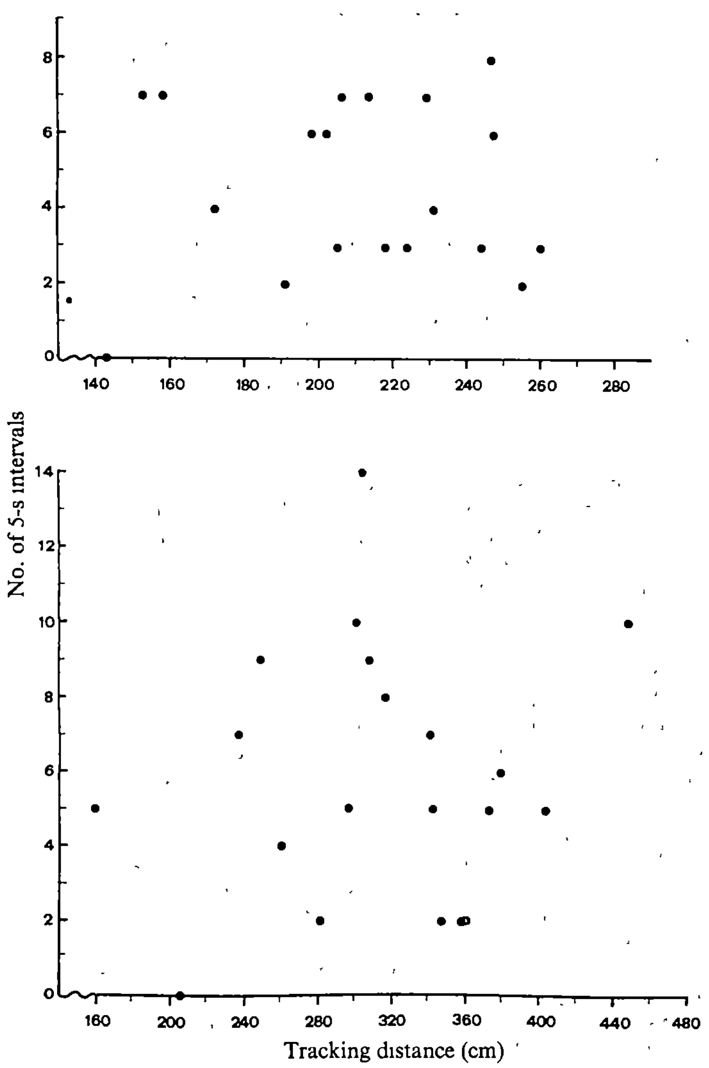

Fig. 3 Relationship between tracking distance during and number of backing-turning responses after presentation of the dummy for 40-(*a*) and 80-s intervals (*b*) The abscissa represents the tracking distance covered by individual larvae; the ordinate refers to the number of 5-s units for which a given individual displayed backing-turning responses during the first 3 min after removal of the dummy. For both experiments, the Spearman rank correlation test failed to show a significant relationship between the larvae's tracking distance and the number of backing-turning responses subsequently carried out. *a*, $r_s = 0.12$; *b*, $r_s = 0.0038$

backing and turning occurred, (3) the backing distance, (4) the amount of angular turning, and (5) the extent to which the animals had changed the orientation of their head and body as the outcome of the entire performance of the stereotyped response (either by turning repeatedly in the same direction, or by making clockwise as well as anticlockwise turning motions) (Fig. 2*b*).

During the two longest presentations of the target (40 and 80 s respectively), nearly all subjects followed the dummy until its removal and thus showed the same tracking time (Fig. 2*a*); the same individuals, however, differed considerably with respect to the distance they covered within 40-and 80-s intervals. Figure 3 shows that the correlation coefficient between the larvae's tracking distance and the number of their subsequent backing-turning responses tended towards zero. The intensity or persistence of the backing-turning response seemed therefore to depend on the time of the insects' tracking rather than on the distance they had covered.

Experiments in which the presentation of the dummy exceeded 80 s have demonstrated that after a time of presentation of 90 s or more the larvae cease to approach the prey and begin to back up and turn away from it, as if the dummy were no longer in their visual field. Thereafter, the insects might oscillate for several minutes between bouts of positive fixation, often combined with approach reactions, and short spells of negative backing-turning away from the dummy. Thus, the removal of the dummy eliminates the tracking command and therefore facilitates and synchronises the appearance of a whole sequence of backing-turning motions; it is, however, by no means a necessary condition for the release of that stereotyped pattern of locomotion.

Further experiments and observations involving the presentation of artificial as well as natural prey indicated that backing-turning occurred only during or after the presentation of a prey stimulus which had previously elicited an approach response. This stereotyped response seemed therefore to be a consequence of a specific activity, and not simply a result of the stimulus which had released this activity. Nevertheless, proprioceptive information resulting from predatory locomotion did not seem to have any influence on the occurrence of backing-turning. For instance, backing-turning movements occurred mainly after the pursuit had reached the stage of walking and creeping. Some insects, however, displayed (weak) backing-turning responses after the dummy had been presented for only a short time; in these cases, due to the brevity of presentation, the tracking responses took the form of swimming movements only. Furthermore, interpolated acts —such as striking and eating a real prey between repeated presentations of the dummy of short duration—did not reduce the cumulative after effect on backing-turning exerted by previous tracking movements. Finally, metabolic changes resulting from the energy consumption[8] during the tracking activity likewise seemed to have no influence on the occurrence of the stereotyped pattern of locomotion. Among other things, the priming effect of tracking depended on the insects' tracking time rather than on the tracking distance, the latter parameter being correlated with the larva's energy consumption. Thus, by analogy with Blest's[5] explanation of the priming effect which flying exerts on the stereotyped settling movements in hemileucine moths, the instigation of the backing-turning pattern may be explained by time-dependent central nervous after effects originating from the activation of those mechanisms which control tracking.

From a functional point of view one may consider that backing-turning has the following effects: (1) it stops any futile attempt on the part of the insect to catch a prey able to escape; (2) it reduces the probability that such a prey will be rediscovered after its momentary disappearance; (3) as direct observation of the larvae's hunting behaviour in the presence of living prey has shown, turning favours the detection of a new prey; (4) finally, the loss of contact with an escaping target reduces the insects' energy consumption as well as the danger that their tracking movements may attract their own enemies.

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Received November 21, 1975; accepted January 30, 1976.

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Modulation of B-cell interactions by T cells

INTERACTION between different lymphoid and non-lymphoid cells is a well established requirement for the development of the immune response *in vivo* and *in vitro*[1–3]. From this it follows that ultimately the regulation of the immune response must depend on the nature and frequency of such cell interactions.

Recently, in the process of examining suspensions of lymph node cells, stained with fluorescein isothiocyanate (FITC)-labelled rabbit anti-mouse immunoglobulin and obtained from mice partially depleted of T cells after treatment with anti-lymphocyte antiserum (ALS), a very high frequency of cell pairs was noticed, in contrast with control cell suspensions. We have followed up that observation and examined lymph node cell suspensions from control and ALS-treated CBA and C3H/Bi mice, and mice treated with ALS and reconstituted with thymocytes, in order to analyse the frequency, characteristics and kinetics of cell pairing. We found a higher frequency of pairs in the ALS-treated mice both in the cell suspensions stained with the FITC-labelled antiserum and in unstained suspensions prepared immediately after removal of the lymph nodes, washed twice, resuspended to a concentration of 1.2×10^9 per ml and examined in a haemocytometer chamber within 30 min of dissection (Table 1).

Whether counted in slides after staining with FITC-labelled immunoglobulin or counted in haemocytometer chambers after washing, the proportions of pairs found in ALS-treated suspensions were consistently higher (9.44%±2.02:6.92%±0.88) than in the respective controls (2.44%±1.02:1.31%±0.38). Moreover, the high frequency of pairs in the ALS-treated suspensions was counteracted by reconstitution with thymocytes (2.97%±0.34:3.25%±1.26).

In addition to the quantitative differences between the cell suspensions, qualitatively, the nature of the cell pairs examined also differed (Table 2). After ALS treatment, the increase in the numbers of pairs was due chiefly to homologous B-cell pairs (4.47%±0.95) and heterologous pairs (3.74%±1.83). Only a small proportion of pairs (1.07%±0.90) consisted of two unlabelled lymphoid cells, presumably T cells. Larger aggregates of cells, consisting chiefly of B cells, were also present in the smears of the ALS-treated lymph node cell suspensions, examination of these under phase contrast, revealed that, although some of the unlabelled cells were clearly lymphoid, some heterologous pairs consisted of one macrophage and one B cell. In the control cell suspensions most of the few existing pairs consisted of two unlabelled lymphoid cells (1.71±0.81). Reconstitution with thymocytes resulted in a low frequency of pairs, comparable with that observed in the controls, that is, most of the existing pairs (1.33±0.84) consisted of two unlabelled lymphoid cells.

Table 1 Percentage of cell pairs in lymph node suspensions of ALS-treated and control mice, and ALS-treated mice reconstituted with thymocytes

Treatment	No of mice	Pairs (%)	
		Slide	Haemocytometer
—	6	2.44±0.83	1.31±0.38
ALS*	12	9.44±2.02	6.92±0.88
ALS ± thymocytes†	4	2.97±0.34	3.25±1.26

Cells were examined unstained (haemocytometer) or after staining with an FITC-labelled antiserum (slide) The ALS-treated mice received 1.5 ml of ALS (Searle) at 2–3 months old, distributed in 3 subcutaneous injections of 0.5 ml given on alternate days before killing. This induced moderate decreases in the proportion of T cells (untreated: 72.8±8.14; ALS· 54.3±11.5) in the mesenteric and inguinal lymph node suspensions.

ALS-treated thymocyte-reconstituted mice received 1 ml or 0.5 ml ALS on day 0, on day 1 they received an intraperitoneal injection of 3.6×10^7 syngeneic thymocytes; and they were killed on day 2.

Cells examined in the haemocytemeter were adjusted to 1×10^6 ml^{-1} in MEM or medium 199.

Cells examined in microscope slides were stained with rabbit anti-mouse immunoglobulin and counted by the standard direct immunofluorescence technique. Minimum of 300 cells counted.

Means ± s.d.

*$P < 0.00001$.

†$P < 0.5$.

Table 2 Nature of cell pairs found in lymph node suspensions* of ALS-treated and control mice, and ALS-treated mice reconstituted with thymocytes

Group	Pairs (%)	−/−	−/+	+/+
Control	2.44±1.02	1.71±0 81	0.3 ±0 35	0.57±0.48
ALS	9.44±2.02	1.07±0.90 ($P < 0.25$)	3.74±1.83 ($P < 0.005$)	4 74±0.95 ($P < 0.00001$)
ALS + thymocytes	2.97±0.34	1.33±0.84 ($P < 0.61$)	0.94±0.04 ($P < 0.07$)	0.71±0.54 ($P < 0.77$)

*Stained with an FITC-labelled rabbit anti-mouse immunoglobulin antiserum. See legend to Table 1. −/−: Homologous, surface IgG negative; −/+: heterologous, consisting of 1 labelled and 1 unlabelled cell; +/+: homologous, surface IgG positive.

Mean ± s.d.

Finally the kinetics of pair formation in the control and ALS-treated populations was studied in Couette viscometer chambers where cells are allowed to collide in well controlled conditions of constant shear rate, viscosity and temperature (Table 3). Although some variation was observed between control and experimental groups, in individual experiments the ratios between the ALS-treated and control suspensions within each experiment were similar. The numbers of pairs in the partially T-cell depleted suspensions were always higher than in the controls (reflected by ratios > 1); at 7 min the pairs in both suspensions tended to disaggregate (reflected in the ratio nearest 1, 1.43) but at the later times, 21 and 28 min, reaggregation was observed at higher levels in the partially T-cell depleted suspensions. In conclusion, the present results indicate that partial T-cell depletion leads to an increase in the frequency of heterologous and B–B direct cell interactions, which can be reversed by reconstitution with thymocytes. It has been shown previously that both T and B lymphocytes release factors capable of diminishing the adhesion of the unlike cell type (B or T) *in vitro*[5]. This study is the first definitive indication that such factors must be operating *in vivo*. The increase in the numbers of direct B-cell interactions must occur as the consequence of a decrease in the concentration of T factor; whether the partial reduction in the numbers of T cells is sufficient to lead to that decrease or whether ALS has an additional inhibitory action on the release of the T-cell factor needs to be clarified. Whatever the exact mechanism, the effect was reversed by the *in vivo* administration of thymocytes.

The relevance of these observations to current theories of the mechanism of T-cell regulation of the immune response is obvious. They clearly offer an attractive cellular explanation of the finding of enhanced antibody production in partially T-cell depleted animals[6] (reviewed in ref. 7). If, in a partially T-cell depleted population of lymph node cells obtained in the absence of a deliberately administered antigen the chance of direct interactions between different cell classes increases so considerably, addition of antigens, especially thymus independ-

Table 3 Kinetics of cell aggregation

Time (min)	Ratio* (ALS treated/control)
0	3.31±1.58
7	1 43±1.00
14	2.04±1.08
21	2.11±0.73
28	2.17±1.10

*Mean ± s.d. (5 experiments)

Aggregation was measured in Couette viscometers[4] set at shear rates of 10 s^{-1}, at 37 °C.

ent, will meet with the most favourable physical conditions for specific information to be passed economically between interacting cells. The requirement of cell aggregation for immunostimulation to occur has been demonstrated in other *in vitro* studies of cell interaction[8]. Moreover, we have now shown that administration of thymocytes reduces the number of B–B cell interactions. The most likely functional repercussion of such an effect *in vivo* would be suppressed or defective antibody production as a direct result of diminished B-cell activation. The suppressive effect of thymocytes administered to mice immunised with SSSIII has been elegantly demonstrated by the extensive work of Baker and his associates[9]. The question of how T cells exert a regulatory role of B-cell function has been the subject of much discussion. The mechanism we have now been able to visualise, strikes us as a simple and ingenious control device which could be operating in most experimental systems *in vivo* and *in vitro* in which helper versus suppressive effects have been described[10].

This work was supported by a grant from the Wellcome Trust.

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Received December 11, 1975; accepted February 6, 1976.

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Effect of interferon on lymphocyte transformation and nuclear antigen production by Epstein–Barr virus

THE Epstein–Barr virus (EBV) can transform human and simian lymphocytes into blast cells which are then capable of indefinite growth *in vitro*[1–4], with 85–100% of the cell population expressing the EBV-induced nuclear antigen (EBNA). Biological[5–7] and molecular[8] evidence shows clearly that there are at least two types of EBV strains, one of which (P3HR-1 EBV) does not transform human lymphocytes[5,6] and seems to possess approximately 15% more DNA sequences than the highly transforming strain (B95-8 EBV)[8]. Furthermore, by infecting cells of the established EBV genome-negative BJA-B line[9] it was also possible to show that whereas B95-8 EBV induces only EBNA in these cells, P3HR-1 EBV induces both EBNA and early antigen (EA)[7]. Thus, by using cells of BJA-B line, it now seems possible to investigate further several known viral functions[10]. We report here the results of a study in which we found that lymphocyte transformation by B95-8 strain of EBV could not be prevented by interferon. As shown below, this finding is further supported by the observation that in spite of the presence of interferon in very high doses (that is, up to 10^4 U ml^{-1}), EBNA can still be produced in a fraction of the EBV-infected BJA-B cells.

It is already known that interferon may not only inhibit virus replication but also cell transformation by viruses[11–14]. Herpes viruses in general, however, seem to be relatively insensitive to the antiviral effect of interferon (see ref. 15 for review). The effect of interferon on cell transformation by EBV and other herpesviruses has not yet been described. Moreover, the effect of interferon on the expression of EBNA has not been investigated.

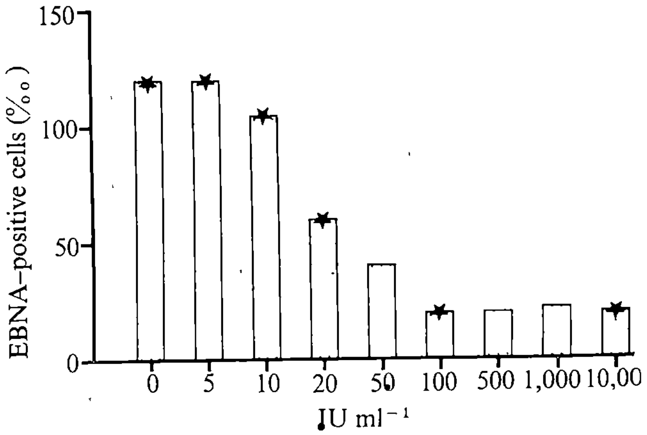

Fig. 1 Effect of interferon on EBNA production in BJA-B cells infected with B95–8 strain of EBV. Interferon was added to the culture medium 24 h before EBV infection. Interferon at these same concentrations was also added to the new medium used after infection, and the cells incubated for 72 h at 37 °C. Smears were prepared as described in Table 1. Whereas the results at 50, 500 and 1,000 IU are from single experiments, all others represent means from two separate experiments.

The experimental approach and the data on the effect of interferon on lymphocyte transformation are given in Table 1. Pretreatment of cells for 24–72 h with the interferon even at doses as high as 10^4 IU ml^{-1} did not inhibit lymphocyte transformation by EBV. No significant differences were observed in terms of time required for lymphocyte transformation by EBV (that is, development of a lymphoblastoid cell line · LCL) irrespective of the various interferon concentrations used. 85–97% cells from the transformed cultures contained EBNA when tested 3 weeks after infection. It is now well known that the presence of EBNA in the cells of a continuously growing lymphocyte culture is the best available evidence for EBV-induced transformation[5,10]. Several attempts to try to quantitate any effect of interferon on lymphocyte transformation by EBV were unsuccessful, since plating of EBV-infected CBL gave negative results in our hands (that is, EBV-infected CBL did not generally grow or stay viable for more than 5–7 d in semi-solid media, even in the presence of conditioning medium). We then decided to determine if CBL were sensitive to the anti-viral effect of interferon. Thus, using herpes simplex viruses we found that interferon can protect a large fraction of the cells against the cytocidal effect of these viruses (Table 2).

Fig. 2 Effect of interferon (●) on the kinetics of EBNA expression in EBV-infected BJA-B cells. ○, EBV alone. Cells were first cultured for 24 h in medium containing interferon (100 IU ml^{-1}) and infected as described in Table 1. The new culture medium added after EBV infection also contained interferon at this same concentration. Results represent means of two separate experiments

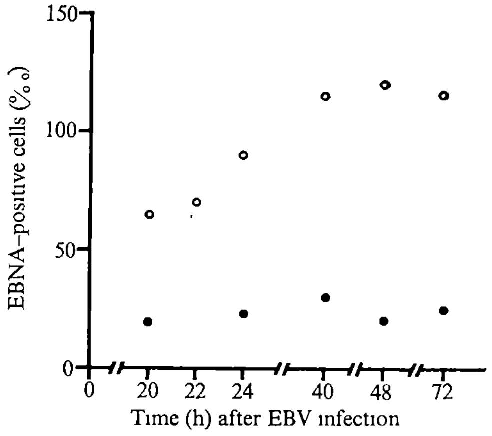

Table 1 Transformation of CBL by the B95–8 strain of EBV in the presence of interferon*

	24, 48 and 72 h before infection	Interferon added simultaneously with EBV	12, 24 and 48 h after infection	No interferon
Time (d) required for transformation†	10–16	10–16	10–16	10–16
% EBNA-positive cells at 4 weeks	> 85	> 85	> 85	> 85

Lymphocyte transformation assays were carried out using human umbilical cord blood lymphocytes (CBL). CBL separation, culture, virus preparation and titration, EA and VCA (virus capsid antigen) staining procedures as well as tests for EBV specificity, were as described previously[5]. Two different preparations of human interferon, one concentrated and the other partially purified, containing 7.5×10^5 interferon units (IU) ml^{-1} (10^4 IU per mg protein) and 2.5×10^6 IU ml^{-1} (10^5 IU per mg protein) respectively, produced in Sendai virus-treated leukocytes, were supplied by Dr Kari Cantell (Central Public Health Laboratory, Helsinki). The procedures for interferon preparation, concentration and titration were as described previously[16]. The anti-viral activity of the interferon preparations was confirmed in our laboratory by a microassay system[17] on human embryo fibroblasts using Sindbis virus as challenger.

For EBNA induction in BJA-B, 10^6 cells were pelleted by a low speed centrifugation and the pellet resuspended in 0.5 ml of a virus dilution (known to be capable of inducing EBNA in about 12% of cells, collected 48 h after EBV infection). The cells were then gently dispersed and the mixture incubated for 1 h at 37 °C in a CO_2-humidified incubator. Growth medium (RPMI 1640 containing 10% foetal bovine serum and antibiotics)[9] was then added to bring the cell concentration to 10^5 cells ml^{-1} and this suspension was dispersed in culture tubes which were then kept at 37 °C for a desired period of incubation. The anti-complement immunofluorescence method[18] was used for EBNA staining but with the following modifications. JM reference serum[18] with anti-EBNA+VCA+EA antibody composition, diluted 1:5 in phosphate-buffered saline (PBS, *p*H 7.2) was used as common source of anti-EBNA antibody and complement; fresh JM serum in 0 2 ml aliquots was kept frozen at −90 °C until use. Smears with about 10^6 cells per coverslip were air dried at room temperature and fixed for 3 min in a 1:1 mixture of chilled (−20 °C) acetone–methanol and kept frozen at −20 °C until use. For EBNA reaction, fixed smears were incubated at room temperature for 45 min with fresh JM serum diluted to 1:5 in PBS, then washed twice with the latter and incubated for 45 min at room temperature with anti-human B_1C/B_1A FITC conjugate (Hyland Laboratories) at 1:20 dilution. The smears were subsequently washed (in PBS) three times, counterstained with Evans blue, washed three times and mounted on a slide in a 1:1 mixture of glycerol–PBS. Controls included (*a*) uninfected BJA-B cells, (*b*) EBV genome-positive Raji cells, (*c*) heat-inactivated (56 °C per 30 min) JM serum and (*d*) fresh EBV–0 serum, without detectable anti-EBV antibody, derived from a healthy EBV-seronegative donor.

*First, CBL of four different donors were used for these experiments. Interferon concentrations tested were 10^2, 10^3 and 10^4 IU ml^{-1}, respectively, and maintained by adding interferon to the new medium at every change. The EBV preparation used here had a transforming titre of $10^{5.5}$ $log_{10}TDC_{50}$ ml^{-1}. Infection was carried with 0.1 ml virus suspension per 10^6 CBL. The experiments were then repeated with CBL of two other donors with a new, partially purified interferon preparation (see above). At least two cultures (tubes and flasks) per donor were used in each of the above experiments.

†Transformation was confirmed by the criteria defined previously[5]. No transformation was observed in uninfected cultures, and the transforming activity was neutralised by pretreating the virus suspension with reference JM serum containing anti-EBV antibody. Serum from EBV-seronegative donor did not neutralise this transforming activity.

In preliminary experiments using BJA-B cells and CBL, we had found that in both cell types interferon had a similar inhibitory effect on the induction of EBNA by EBV. We therefore decided to quantify this effect only in the EBV genome negative BJA-B cell line. In BJA-B cells, the expression of EBNA could not be completely inhibited by interferon, even at doses as high as 10^4 U ml^{-1}. There was no detectable interferon effect (reduction in number of EBNA-positive cells) at 5 IU ml^{-1} but a considerable reduction was observed at doses over 10 IU ml^{-1}. Doses of 10^2–10^4 IU ml^{-1} gave similar results (Fig. 1). In preliminary experiments during the present study we found that in our experimental conditions the number of EBNA-positive BJA-B cells reached its maximum 40–48 h following EBV infection. Therefore, we investigated the effect of interferon on the appearance of EBNA up to 76 h after EBV infection. Figure 2 shows that a considerable reduction in the number of EBNA-positive cells was consistently detected in the presence of interferon in all the samples collected 20–72 h after EBV infection. Two other single experiments carried out using a more concentrated virus preparation (capable of inducing EBNA in about 20% of BJA-B cells), as well as interferon treatment of the cells beginning 1 week before EBV infection, also gave similar results. From these findings, it is difficult to understand why the anti-viral (EBNA) effect of interferon is effective only in a fraction of BJA-B cells. It is, however, possible that a subpopultaion of these cells is interferon resistant. In EBV-infected CBL cultures such a resistant cell population would outgrow the protected cells during the period (10–16 d) up to when the transformation is confirmed, and would thus explain the observed interferon resistance of CBL transformation by EBV. Note, however, that in other virus–cell systems interferon resistance in a fraction of virus populations[19] and/or a fraction of virus-infected cells[20] have been considered in the past as possible explanations for such a phenomenon.

Interestingly, it was previously found that interferon was unable to block the enhanced expression of EBV-induced antigens by hydrocortisone in EBV genome-carrying lymphoblastoid cell lines[21]. Note, however, that interferon has an inhibitory effect on cell transformation by other well known DNA viruses such as SV40 and adenoviruses[12–14]; this effect may, however, vary in degree according to the cell system and the virus used[12,13]. On the other hand, the effect of high interferon concentrations (10^4 IU ml^{-1}) on cell transformation as used in the present study was not tested against SV40 and adenoviruses[12,14]. Interestingly, the effect of interferon on EBNA production seems to be similar to its effect on T-antigen synthesis by SV40 and adenoviruses. In this respect, it was suggested that the expression of SV40 genome is sensitive to the action of interferon only if the SV40 genome is not integrated into the cell genome[13,22]. Since the question of the integration of the EBV genome into the cellular genome has not yet been completely resolved, it may be very important to transform CBL in the presence and/or absence of interferon and examine the LCL thus obtained for the relation of EBV genome to cellular genome in terms of integration.

Table 2 Sensitivity of CBL to the anti-viral action of interferon as tested against cytocidal effect by herpes simplex viruses types 1 and 2*

Virus type	Virus dose ($log_{10}TCD_{50}$ ml^{-1})	Interferon concentrations (U per 0.2 ml per 200,000 cells) 0	10	100	1,000
HSV 1	10^2	12†	38	70	71
	10^3	10	20	30	39
HSV 2	10^2	24	20	65	63
	10^3	10	17	40	42
Control (CBL cultures)	—	92	99	99	97

*CBL from a single donor were preincubated with interferon for 48 h. Virus was then added to these cultures which were further incubated for 96 h at 37 °C, and the cells examined for viability by Trypan blue dye exclusion test.

†Figures represent percentage of viable cells in each of the above conditions.

We thank Dr Kari Cantell for interferon preparations and for comments on the manuscript, and Michèle Leyritz for carrying out the interferon titration in the microassay system. Supported by the Cancer Research Society Inc. (Montreal), the National Cancer Institute of Canada and the MRC of Canada, (of which J.M is a Scholar) W.L. was supported by the Deutsche Forschungsgemeinschaft.

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Growth of bacilli on methyl-α-D-glucoside

METHYL-α-D-GLUCOSIDE (αMG) is an analogue of glucose that is used extensively for studies of glucose transport in microorganisms[1,2]. This use implies that the analogue enters the cell and is phosphorylated[2], but is not further metabolised It is known[2] that *Escherichia coli* do not grow on αMG and Freese *et al*[3] reported that *Bacillus subtilis* (strain 60015) can grow on αMG only after a long lag period: the subsequent growth rate being very slow. This may not, however, be typical of bacilli since here we report several strains of bacilli which grow readily on αMG.

E. coli (KL16), *B. stearothermophilus* var *non-diastaticus*, *B. subtilis* (Marbourg) and *B. licheniformis* (A5) were grown on nutrient broth and then inoculated into salts medium (for *E. coli* see ref. 4; for *B. stearothermophilus* ref. 5; for the others ref. 6) containing 25 mM of one of the following sugars as the sole carbon source: αMG, 2-deoxyglucose, cellobiose, melibiose, maltose, lactose, sucrose or glucose. The cultures were incubated aerobically overnight (*B. stearothermophilus* at 55 °C, the others at 37 °C) to establish which sugars supported the growth of these organisms. The results show that whereas *E coli* does not grow on αMG (nor sucrose, nor cellobiose), all of the bacilli tested grew readily on these carbohydrates. All organisms tested grew readily on glucose and maltose, but not on 2-deoxyglucose.

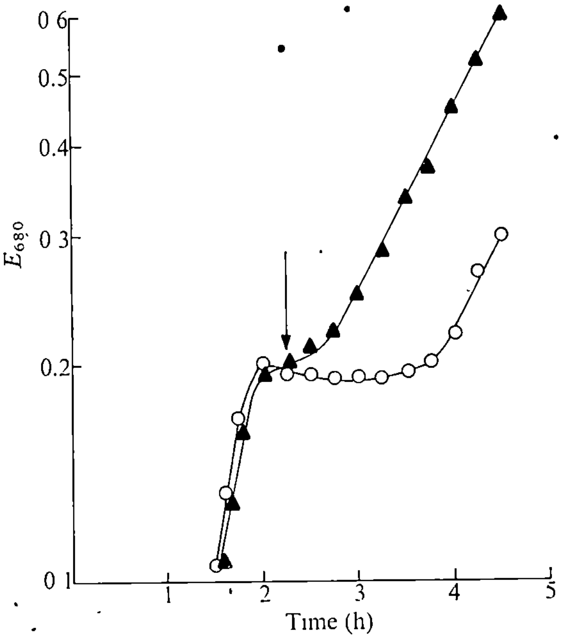

Fig. 1 Growth of *B licheniformis* on glucose (○), and maltose (▲), and subsequent growth on αMG (2 mM added as indicated by the arrow)

In view of the fact that the bacilli grow on αMG, the growth rates and yield of cells were compared from bacilli growing on αMG and on glucose *B. stearothermophilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis* were grown on nutrient broth and then inoculated into salts medium containing either 2 mM glucose or 2 mM αMG. The cultures were incubated aerobically at 37 °C and growth followed by measuring the absorbance of the cultures at 680 nM. When both of the cultures had just run out of substrate, αMG to 2 mM was added to the culture that had grown on glucose and the time before growth of the culture began on the new substrate was recorded as the lag period for the organism to adapt from metabolising

Table 1 Growth of *B. stearothermophilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis* on glucose and αMG

Organism	Incubation temperature (°C)	Glucose (2 mM) Doubling time (min)	Glucose (2 mM) ΔE_{680}	αMG (2 mM) Doubling time (min)	αMG (2 mM) ΔE_{680}	Lag period (min)
B licheniformis	37	50	0.315	90	0.405	120
B. megaterium	37	45	0.420	70	0.520	90
B. subtilis	37	60	0.390	120	0 460	50
B stearothermophilus	55	50	0.285	70	0.365	75

The organisms were first grown on nutrient broth and then inoculated into salts medium containing 2 mM of either glucose or αMG. When the glucose cultures had exhausted the added glucose, 2 mM αMG was added. The interval before exponential growth resumed was recorded as the lag period between the metabolism of glucose and that of αMG.

glucose to metabolising αMG. The results (Table 1) show that all the bacilli grow more slowly on αMG than on glucose but that the yield of cells from each substrate is about the same. Furthermore, there is a lag period between a bacillus metabolising αMG and metabolising glucose. In contrast, bacilli grown on maltose will immediately grow on αMG with no corresponding lag (Fig. 1). This suggests that bacilli possess a methyl-α-glucosidase which could be similar to that demonstrated in yeast (*S. cerevisiae*) which was shown to be specific for αMG in this organisms, and was coinduced by growth on maltose[7-9].

The results of this study show that bacilli grow readily on αMG, the metabolic enzyme (possibly methyl-α-glucosidase similar to that in yeast) being induced by growth on maltose and αMG. This means that αMG can only be used as a non-metabolisable analogue of glucose in bacilli if the organism has not been grown on maltose, and providing αMG is used for only a short period of time.

This work was supported by the Science Research Council through a grant to Professor H. L. Kornberg. We thank Professor N. Grossowicz for a gift of *B. stearothermophilus* var. *non-diastaticus*.

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Received March 6, 1972; accepted February 3, 1976.

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Methyl-α-D-glucoside uptake and splitting by a thermophilic bacillus

THE presence and properties of a phosphoenolpyruvate (PEP)-dependent phosphotransferase system in a thermophilic bacillus has been described recently by Harris and Kornberg. The formation of the glucose and methyl-α-D-glucopyranoside (αMG) uptake and phosphorylation is induced by glucose[1].

In the course of a study on the transport of glucose and αMG in the same thermophilic bacillus[2] we noticed that growth occurred in a minimal medium supplemented with αMG as the sole carbon source. The generation time at 58 °C was found to be 52 min. The growth rates in presence of αMG, glucose and succinate are given in Fig. 1. In addition to αMG, other α-glucosides, that is, maltose, sucrose, trehalose and turanose can also serve as sole carbon sources.

Washed suspensions of αMG grown cells incorporated ^{14}C-αMG into the macromolecular constituents of the cell (Table 1). Almost no ^{14}C-αMG was incorporated with suspensions of glucose-grown cells. Cellular extracts of αMG and maltose-grown cultures which were allowed to take up ^{14}C-αMG showed by thin-layer chromatography and autoradiography several spots, representing, presumably, different degradation products of the sugar; whereas extracts from cells grown on glucose revealed only a single spot in addition to the residual αMG (Fig. 2), corresponding to an αMG phosphate (to be published).

The above results demonstrate the presence of an enzyme system in the thermophilic bacillus responsible for the hydrolysis (and metabolism) of αMG. A study was carried out to identify the enzyme(s) and conditions for their activity, using intact cell suspensions and *p*-nitrophenyl-α-D-glucopyranoside (PNPG) as substrate. PNPG hydrolysis was linear with time for about 30 min and cell concentrations up to at least 0.25 mg dry weight.

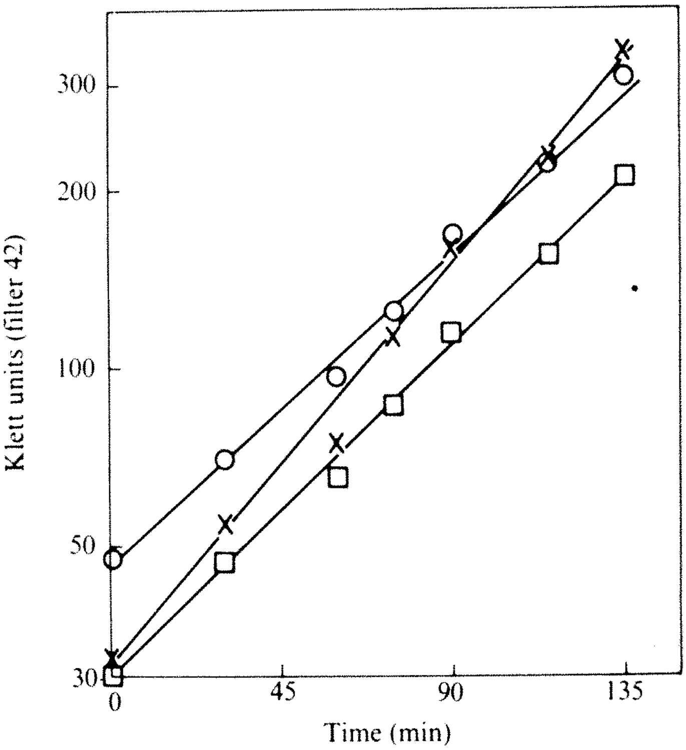

Fig. 1 Growth of the thermophilic bacillus at 58 °C on various carbon sources; 0.5% αMG (○), 0.5% Na-succinate (□) and 0.5% glucose (×). The tyrosine–leucine auxotroph and the TL-minimal medium were used; growth conditions were as described elsewhere[3].

High αMG-hydrolysing activity was induced by αMG or maltose; turanose and trehalose were much poorer inducers, while sucrose was completely inactive (Fig. 3). Higher levels of induction were obtained in rich medium than in the minimal medium.

In contrast to the ability of induced intact cells to split PNPG or αMG, no activity was found when using cell-free preparations. Moreover, impairment of the integrity of the cell membrane (by treatment with toluene, sonic oscillation or lysozome) resulted in the loss of the α-glucosidase activity. Addition of phosphoenolpyruvate (PEP), glucose-6-phosphate or adenosine triphosphate (10 μmol each) to the cell-free system failed to restore enzymatic activity.

It was shown by Harris and Kornberg[1] that glucose induces the uptake and phosphorylation of glucose and αMG by cultures of the thermophilic bacillus grown in either acetate or succinate. As shown in Table 2 the PEP-dependent phosphorlyation of ^{14}C-αMG was induced not

Table 1 ^{14}C-αMG incorporation into the macromolecular fraction of the thermophilic bacillus

Carbon source for growth	c.p.m. per sample Whole cells	 5% TCA precipitate
Na succinate+glucose	4,700	360
Na succinate+αMG	21,045	11,680
αMG	22,076	12,420

Cells were grown at 58 °C in TL-minimal medium[3] supplemented with the indicated carbon source. Duration of uptake of ^{14}C-αMG (1×10^{-4} M; specific activity, 3 μCi μmol^{-1}), in medium lacking carbon source, was 10 min. Thereafter, two 0.2-ml samples, 0.1 mg dry weight of cells each, were taken for determinations of the total uptake and radioactivity in cold TCA (5%) precipitate[3]. Uptake refers to the radioactivity accumulated in cells, and thus includes both label of the sugar and metabolic products derived from it.

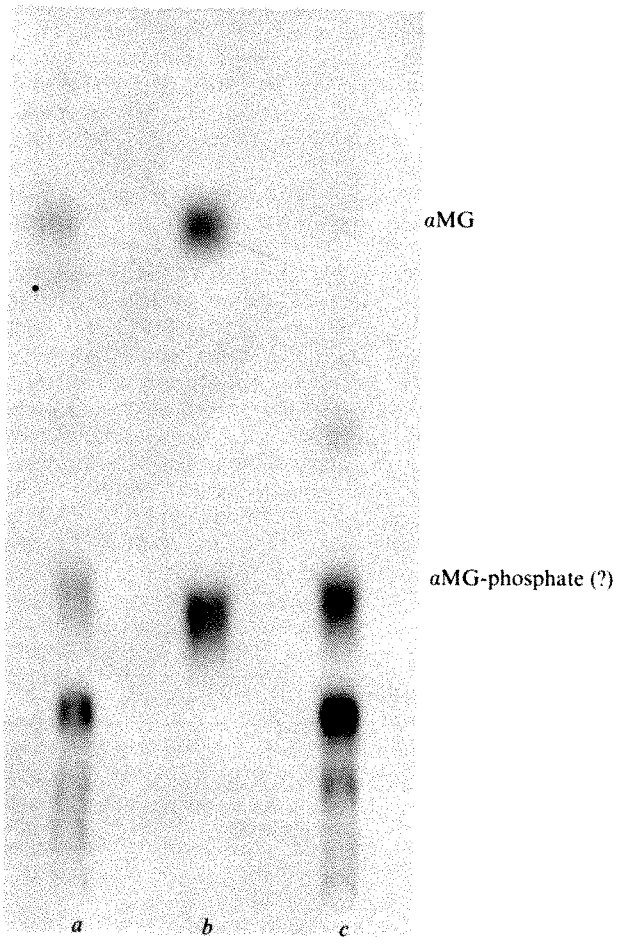

Fig. 2 Metabolism of ^{14}C-αMG. ^{14}C-αMG (2×10^{-4} M, specific activity 3 μCi μmol^{-1}) was added to washed suspensions (0.3 mg dry weight per ml) from *a*, αMG; *b*, glucose and *c*, maltose grown cultures; total volume 4 ml. After 1 min at 58 °C the cells were collected on membrane filters, washed and immediately plunged into boiling 1 mM Tris-HCl buffer at *p*H 9.0 and held for 6 min. Cell debris was removed by centrifugation. The supernatant fluid was concentrated by lyophilisation and aliquots were chromatographed on cellulose-coated sheets using ammonium acetate 1 M (*p*H 4.0)–95% ethanol (2 : 5 v/v) solvent system. The autoradiograms were observed after 7 d of exposure.

only with glucose, but also with αMG. According to these investigators[1] the uptake of αMG by intact cells of the thermophilic bacillus was directly correlated with the activity of the phosphotransferase, which catalyses the PEP-dependent phosphorylation of αMG. It seemed, therefore, worthwhile to test the possibility that αMG induces its own transport. Figure 4 shows that induction of αMG transport could indeed be achieved not only by glucose but also by αMG; the differential rate of the synthesis of the

Table 2 Induction of αMG phosphotransferase activity

Incubation mixture	Cell-free extract from cultures grown on Succinate	Succinate+glucose	Succinate+αMG
Complete	1,500	10,400	6,170
Without PEP	910	1,015	330
Without PEP; +ATP	1,020	2,030	505

Cultures were grown in TL-minimal medium with the indicated carbon sources and were collected in the log phase; the cells were washed and resuspended in 0.05 M potassium phosphate buffer (*p*H 7.2), treated with lysozyme (0.1 mg ml^{-1}) and then sonicated for 60 s using the MSE sonifier. The sonicates were centrifuged for 10 min at 750 r.p.m. and the supernatant assayed for ^{14}C-αMG phosphorylation ability at 58 °C according to Freeze *et al.*[4]. The reaction was stopped after 10 min and assayed for DEAE-cellulose-adsorbable radioactivity. Results are expressed in c.p.m. per mg protein.

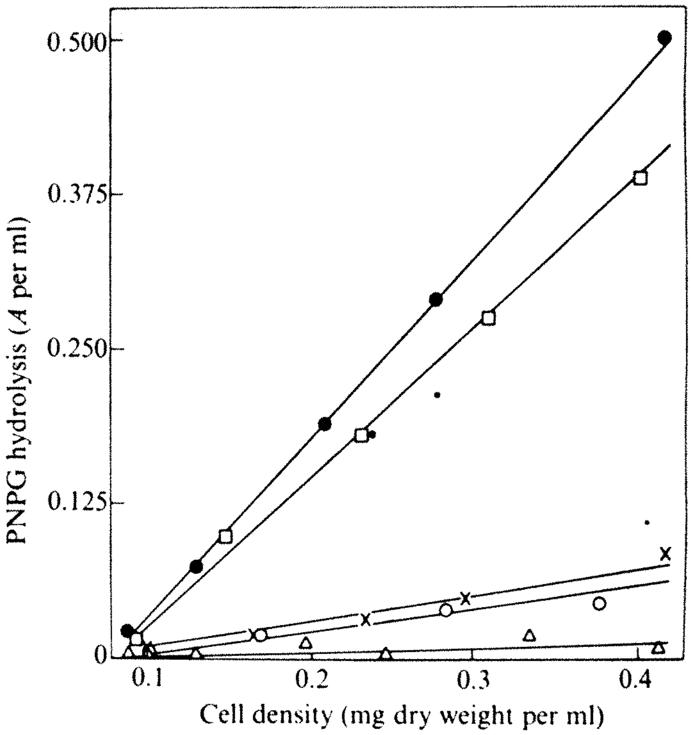

Fig. 3 Induction pattern of PNPG splitting activity by cell suspensions. Cultures were grown at 58 °C in TL-minimal medium supplemented with 10% (v/v) rich medium[3]. For PNPG hydrolysis samples were withdrawn during growth, filtered and the cells resuspended in minimal medium. The reaction was started by adding PNPG (1×10^{-3} M) and terminated by the addition of 1 ml of 2 M Na_2CO_3. The liberated aglycone was determined at 420 nm using a Spectronic 88 spectrophotometer. Controls without substrate were included and the absorbance obtained was subtracted. Carbon source during growth: 0.5% αMG (●); 0.5% maltose (□); 0.5% turanose (×); 0.5% trehalose (○); 0.5% sucrose (△).

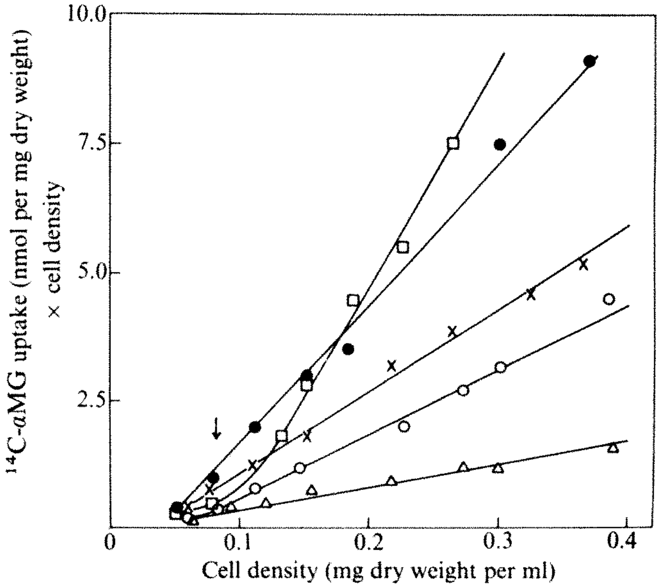

Fig. 4 Induction of ^{14}C-αMG uptake by the thermophilic bacillus. Cultures were grown in TL-minimal medium at 58 °C. Samples were withdrawn during growth, filtered and suspended in the minimal medium lacking the carbon source. Duration of uptake 0.5 min. The ^{14}C-αMG concentration was 5×10^{-5} M, specific activity 3 μCi μmol^{-1}. Carbon sources during growth, 0.5% Na-succinate (△), 0.5% Na-succinate+0.5% αMG (○), 0.5% αMG (×), 0.5% glucose (●), 0.5% Na-succinate+0.5% glucose (□). Arrows show the time of addition of inducer (αMG or glucose) to succinate-grown cultures. For further details see legend to Table 1 and ref. 3.

αMG transport system was, however, much higher in the presence of glucose than in the presence of αMG.

It has already been pointed out by Harris and Kornberg[1] that glucose and αMG differ in their uptake properties. Our results accentuate these differences and show clearly that both the αMG phosphotransferase and the αMG transport system can be induced by αMG and not only by glucose. Although both glucose and αMG induce the uptake of αMG, the αMG-hydrolysing activity is induced by αMG and maltose, but not by glucose. The possibility, therefore, exists that the transport system induced by αMG is different from the one induced by glucose. A study of the specificity of the two inducible transport systems may clarify this point. The existence of an α-glucosidase in various yeast cells is well established[5,6]. In *Saccharomyces cerevisiae* the simultaneous induction of two specific α-glucosidases, that is maltase and αMG-splitting enzyme, by either maltose or αMG have been reported[7].

It remains to be seen whether the α-glucosidases induced by maltose and αMG in the thermophilic bacillus possess different and specific enzymatic activities or they represent a single enzyme with a substrate specificity towards both maltose and αMG.

In summary, our results clearly indicate that the thermophilic bacillus is able to catabolise αMG after it is transported into the cells, although αMG-splitting activity seems rather rare in prokaryotic organisms[8]. This report also demonstrates the ability of αMG to induce its own transport across the cell membrane. The substrates of the α-glucosidase activity, the optimal conditions for the enzymatic hydrolysis, and the reaction products formed, are being studied.

Note added in proof: Similar findings were recently reported with the mesophilic *Bacillus subtilis*[9].

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Received July 14, 1975; accepted February 3, 1976.

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Inhibition of malformin activity by hydroxyproline and restoration by proline

MALFORMIN, (cyclo-D-cysteinyl-D-cysteinyl-L-valyl-D-leucyl-L-isoleucyl)[1], is produced by the fungus *Aspergillus niger* van Tiegh., induces severe malformations on stems and petioles of various plants, and causes root curvature[2]. When ^{14}C-malformin was supplied to cuttings of the bean *Phaseolus vulgaris* L. most of the ^{14}C was found in the wall fraction and was liberated from a protein-like component in the walls as ^{14}C-malformeic acid (SO_3H-malformin) by oxidation[3]. Previous results indicated that malformin inhibited wall synthesis and altered wall composition[4–6]. We recently found that malformin is a potent stimulator of plant growth and inhibits several phytochrome- and ethylene-mediated responses. Because some effects of light and ethylene on plant growth have been attributed to an increase in extensin[8–11], a hydroxyproline-rich protein in plant walls, it was suggested that malformin may alter the synthesis or function of this wall component[7]. Because malformin activity is inhibited by reducing agents[12] and it reacts with sulphydryl compounds[13], involvement of wall sulphydryl groups in the activity of malformin was also proposed[7]. We report here on the effect of hydroxyproline and proline, amino acids important in the synthesis of extensin, on the growth-stimulating and root-curving activities of malformin.

Cuttings from 4-d-old green seedlings of *P. aureus* Roxb., grown in vermiculite, were obtained by excision 7.0 cm below the apical bud. The cuttings were transferred randomly to 12-ml vials containing 6.0 ml of test solution (12 cuttings per vial), and incubated in continuous light (1.35×10^4 erg cm^{-2} s^{-1}, Champion F90T17/w, white fluorescent, 27–29° C). Deionised water was added daily to maintain the volume at approximately 6.0 ml. After 2 d the stem length was measured. Measurements from each vial were averaged and considered as a single determination. The growth increment ratio (GIR) was determined by dividing the growth increment of treated cuttings by that of controls (water treated). Malformin-induced root curvatures were determined by germinating seeds of *Zea mays* L. WF9 × 38-11 for 3 d in Petri dishes containing a sheet of Whatman No. 1 filter paper (9.0 cm) moistened with 3.0 ml of test solution, and counting the number of roots with at least a 90° C curvature from the original direction of growth[2].

Table 1 Effect of L-proline and L-hydroxyproline on growth stimulation and root curvature induced by malformin

Treatment	*Phaseolus aureus* growth stimulation (GIR)*	*Zea mays* root curvature (%)†
H_2O	—	1.4 ± 1.3
Malformin 0.1 μM	1.64 ± 0.06	57.8 ± 11.8
Malformin 0.1 μM, proline 10 mM	1.42 ± 0.04	60.7 ± 10.9
Malformin 0.1 μM, hydroxyproline 10 mM	1.11 ± 0.05	14.3 ± 6.4
Proline 10 mM	0.86 ± 0.06	2.8 ± 2.8
Hydroxyproline 10 mM	0.82 ± 0.05	0.0 ± —

*Growth increment ratio = growth increment of treated cuttings/growth increment of controls (H_2O); ± s.e.

†Percentage roots with at least a 90° curvature; ± s.e.

L-Hydroxyproline markedly inhibited plant growth stimulation and root curvatures induced by malformin, but L-proline had little or no effect (Table 1). Although proline and hydroxyproline both inhibited growth of cuttings when applied alone, only hydroxyproline substantially reduced malformin-induced growth stimulation. Reduction of malformin-induced growth by proline was only slightly greater than that which occurred in the absence of malformin, but that of hydroxyproline was considerably greater. Neither of these amino acids alone stimulated growth or induced root curvatures. L-Leucine, DL-threonine, L-tyrosine, and DL-serine had little effect on malformin-induced growth stimulation, and in earlier experiments L-valine, L-isoleucine, and L-leucine did not inhibit curvatures induced by malformin[12]. We concluded that the inhibition was specific for L-hydroxyproline. Although lower concentrations (1.0, 0.1 mM) of hydroxyproline were effective in inhibiting malformin-induced root curvatures, they had little effect on malformin-induced growth stimulation. Growth changes induced by malformin in corn roots were attributed primarily to effects on cell elongation[6]. In preliminary experiments L-hydroxyproline also reduced the severity of malformations on seedlings of *P. vulgaris* treated with malformin. Malformations on *P. vulgaris* have been described as the result of a change in the orientation of developing cells so that they elongate at an angle to the longitudinal axis of the plant[14].

Table 2 Restoration of malformin activity by L-proline in the presence of an inhibitory concentration of L-hydroxyproline

Treatment	*Phaseolus aureus* growth stimulation (GIR)	*Zea mays* root curvature (%)
H_2O	—	0.5±0.1
Malformin 0.1 μM	1.63±0.04	51.6±4.2
Malformin 0.1 μM, hydroxyproline 10 mM	0.97±0.08	22.9±6.5
Malformin 0.1 μM, hydroxyproline 10 mM, proline 10 mM	1.45±0.05	48.2±4.8
Hydroxyproline 10 mM, proline 10 mM	0.84±0.07	3.1±2.2

Table 3 Proline, hydroxyproline and total amino acid content of cell walls from normal and malformed internodes of *P. vulgaris**

Internode	Total amino acid	Hydroxyproline	Proline	Hydroxyproline / Proline
Normal	21.2±1.6	0.70±0.05	0.68±0.06	1.03±0.17
Malformed	28.6±1.7	2.68±0.12	1.14±0.02	2.35±0.06

*Results are μg amino acid per mg dry weight cell wall ± s.e. Seedlings of *P. vulgaris* cv. Harvester were treated on the apical bud with malformin (10^{-5} M) as described[2]. After 11 d the first internodes above the simple leaves were diced and frozen (−15 °C) until analysed. Frozen material was homogenised for 5 min in 3:1 v/w 0.2 M Tris buffer (*p*H 7.5) and centrifuged at 150*g* for 10 min. The pellet was suspended in 10 ml Tris buffer, homogenised again and centrifuged as described. The cell wall pellet was washed twice with buffer (10 ml) and 3 times with deionised water (10 ml). After each wash the wall fraction was pelleted at 150*g* for 10 min. The wall pellet was washed with trichloroacetic acid (5%, 10 min, 90 °C), cold trichloroacetic acid (5%, 5 min, 10 ml), 95% ethanol (70 °C), ethanol–ether–$CHCl_3$ (2:2:1, 55 °C) and ether–acetone (2:1, 25 °C). After each wash the wall fraction was centrifuged (1,500*g*, 5 min)[18]. The cell walls were air dried, hydrolysed for 24 h by refluxing in 6N HCl (4,000:1 excess HCl) in a CO_2 atmosphere, and the hydrolysate cleared of humin[19] by charcoal and filtration. The hydrolysate was dried *in vacuo* at 60 °C, dissolved in deionised water, and analysed for hydroxyproline[20], proline[21], and total amino acids[22].

Proline rather than hydroxyproline is the usual precursor of bound hydroxyproline in plants[11,15], and the inhibition of cell elongation in the *Avena* coleoptile by hydroxyproline was suggested to result from an inhibition of the formation or utilisation of a hydroxyproline-rich protein in cell walls[16]. Growth inhibitory concentrations (0.5 mM) of hydroxyproline are incorporated directly into coleoptile proteins and proline prevents this incorporation[17]. The ability of proline to restore malformin activity in the presence of inhibitory concentrations of hydroxyproline was examined (Table 2). L-Proline almost completely restored the biological activity of malformin with regard to growth stimulation and root curvatures, although proline itself or in combination with hydroxyproline neither stimulated growth nor induced root curvatures (Tables 1, 2). The amount of amino acid present when proline and hydroxyproline were combined with malformin was double (20 mM) that present when only hydroxyproline was present. To ensure that simply increasing the amount of amino acid did not account for restoration of malformin activity we also assayed malformin activity in the presence of 20 mM hydroxyproline. Malformin activity was not restored.

To determine if malformin alters the metabolism of proline and hydroxyproline we examined the content of these amino acids in cell-wall fractions from normal and malformed internodes of *P. vulgaris*. This tissue was selected because malformin was shown to inhibit wall synthesis and alter wall composition[5], as well as attach to a protein-like component in wall fractions of *P. vulgaris* internodes[3]. On a dry weight basis the amount of total amino acids in wall fractions from malformed internodes was greater than that in similar fractions from normal tissues (Table 3). Malformin induced a marked increase in the amount of hydroxyproline and proline content of wall fractions and increased the hydroxyproline–proline ratio more than twofold. Because malformin seems to have more than one mode of action, stimulates elongation in some systems[7], but inhibits elongation in other systems[2], we are unable to offer an explanation for our results. Since malformin inhibits several phytochrome- and ethylene-mediated responses, the effect of malformin on hydroxyproline-rich wall protein probably differs from that proposed for phytochrome and ethylene[9,10]. But inhibition of malformin activity by hydroxyproline, restoration of activity by proline, and alterations in the hydroxyproline–proline ratio in cell-wall fractions from malformed internodes strongly implicates a role of hydroxyproline-rich protein in the biological activity of malformin.

We thank Mrs P. Phillips for technical assistance.

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Received October 27, 1975; accepted February 3, 1976.

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Parathyroid hormone-responsive adenylate cyclase in induced transplantable osteogenic rat sarcoma

AN apparent paradox prevents accurate assessment of the role of cyclic AMP in the development and maintenance of tumours. Although cell culture experiments indicate that malignant transformation is associated with decreased adenylate cyclase activity and intracellular cyclic AMP levels[1,2], the same is not true of *in vivo* studies with tumours, in which a range of results have been obtained, from low[3] to normal[4] and elevated[5] tumour cell cyclic AMP levels. We report here the development of a transplantable osteogenic sarcoma which has retained through many generations a parathyroid hormone-responsive adenylate cyclase, providing a system which can be used to evaluate the significance of hormone-responsive adenylate cyclase for tumour cell growth and differentiation.

Tumours were induced in Sprague–Dawley rats by injecting carrier-free ^{32}P-orthophosphate according to a schedule which has been shown[6] to lead to a high incidence of osteogenic sarcoma development. After an initial intraperitoneal injection of 1 μCi per g body weight, six subsequent injections of 0.6 μCi per g body weight were given at 2-week intervals. After a lag period of 6–9 months tumours became palpable, usually in the tibiae or femora, in approximately 50% of treated animals. Some microscopic features of primary and transplanted tumours have been described by Geddes-Dwyer *et al.*[7].

All the rats used for tumour induction and subsequent transplantation were derived from one original litter. Tumour transplantation was carried out initially by forcing small pieces of tumour through 60-mesh stainless steel gauze, and injection into the flank of recipients. In later experiments with established tumours, cells were dispersed by collagenase and hyaluronidase digestion, counted in a Coulter counter, and injected (10^6–3×10^6 cells) subcutaneously into the flank. In these conditions tumour transplantation had virtually a 100% success rate. Tumours appeared 2–3 weeks after transplantation and rapidly grew to a large size requiring further transplantation or leading to the death of the animal within 6 weeks.

Although in the early stage of growth the cut surfaces of tumours have a fleshy appearance, calcification eventually occurs in all tumours, frequently with large calcified deposits but sometimes detectable only microscopically. Figure 1 illustrates the microscopical appearance of a transplanted tumour showing early calcification. The mineralisation is not related to necrosis, and takes place on an intercellular ground substance which can readily be shown, with appropriate staining, to consist of collagen and PAS-positive material, presumably mucopolysaccharide. The tumour used for the biochemical and histological experiments described here consisted of fairly uniform cells (Fig. 1) not showing the pleomorphism often seen in human osteogenic sarcoma. In spite of a search through many sections of lightly and heavily calcified tumour, no osteoclast-like cells were seen. Similarly, using electron microscopy the cells were of uniform morphology with prominent polyribosomes and intercellular collagen fibres.

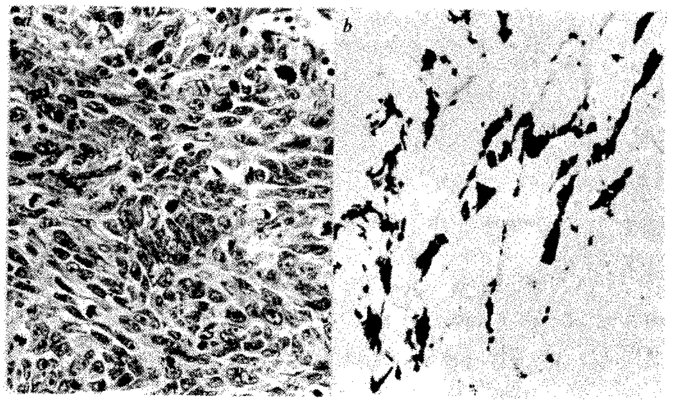

Fig. 1 Section of the tumour stained with haematoxylin and eosin (*a*) and by von Kossa's method (*b*). The tumour cells are slightly elongated, of relatively uniform morphology, and show much mitotic activity. Mineralisation of the intercellular matrix is shown by the dark areas in the von Kossa stained preparation (× 196).

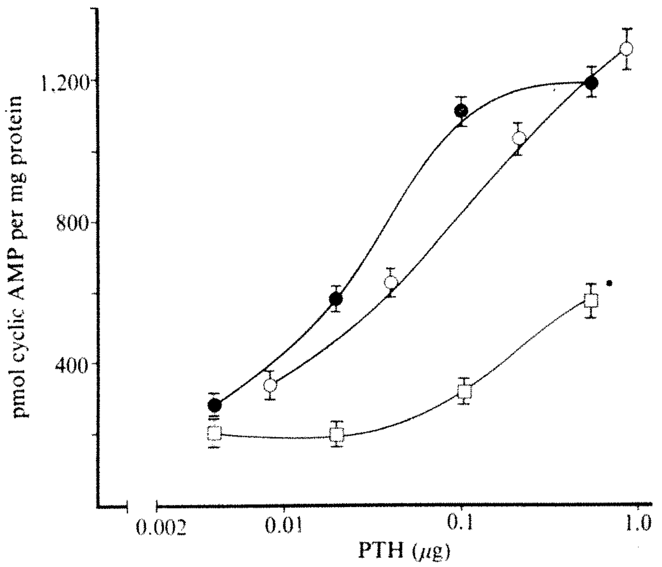

Fig. 2 Adenylate cyclase response of partially purified plasma membranes from rat osteogenic sarcoma on treatment with various preparations of PTH. Membranes were prepared and assay carried out as for Table 1. The hormone preparations used were as follows: ●, synthetic bovine PTH (1-34); □, synthetic bovine PTH (2-34); ○, highly purified bovine PTH, potency 2,500 U mg^{-1} (preparation 72/286, MRC Division of Biological Standards).

In all but those tumours in advanced stages of calcification dispersed cells could readily be prepared, and subcellular preparations made. The remaining experiments to be described were carried out with partially purified plasma membrane preparations, in which the adenylate cyclase activity of the tumour was studied. The response to various hormones is shown in Table 1. The major effect was obtained with parathyroid hormone (PTH) and lesser effects with prostaglandins E_1 and E_2. Isoprenaline, glucagon, and salmon calcitonin stimulated adenylate cyclase to a small extent. The experiment of Fig. 2 illustrates that the interaction between PTH and the receptor in the sarcoma cell membranes resembles that between the hormone and its receptor in rat renal cortex membranes, in that parallel dose responses were obtained with highly purified bovine PTH and the synthetic peptide based on the first 34 amino acids of the hormone's sequence. Furthermore the synthetic (2-34) peptide had approximately the same relative effect in this system as it has been shown to have in rat renal membranes[8]. Oxidised PTH, which is ineffective biologically in other systems, had no stimulatory effect on adenylate cyclase in the tumour cell membranes. Thus the tumour seems to possess a PTH receptor which resembles in certain major features that present in the rat kidney. Although there is some information available regarding the response of bone cell adenylate cyclase to PTH[9], it is not sufficiently complete to enable comparison with the present data.

These experiments were carried out on a tumour which had been transplanted almost 20 times over a period of 2 yr. Several other membrane preparations were examined, derived from other primary osteogenic tumours induced in the same way, and all were found to have a PTH-responsive adenylate cyclase. It is also important to note that in the tumour of this study no detectable change in the nature of the hormonal response took place during intensive study over a period of 6 months, encompassing six transplantations, and furthermore the hormone responsiveness remained constant through all stages of growth of individual tumours.

Changes in adenylate cyclase activity and its hormonal control have been observed in several experimental and

Table 1 Effect of various hormones on adenylate cyclase activity in partially purified plasma membranes from rat osteogenic sarcoma

Treatment	Concentration	Adenylate cyclase activity (pmol cyclic AMP formed per mg protein)*
Nil	—	55.8 ± 2.8
PTH	2×10^{-7} M	175.4 ± 4.9
	2×10^{-6} M	471.4 ± 1.9
Salmon calcitonin	3×10^{-7} M	69.5 ± 1.2
Glucagon	5×10^{-6} M	74.2 ± 1.8
Isoprenaline	10^{-5} M	98.4 ± 2.8
Prostaglandin E_1	10^{-5} M	146.8 ± 4.1
Prostaglandin E_2	10^{-5} M	138.4 ± 2.1
Prostaglandin $F_{2\alpha}$	10^{-5} M	71.2 ± 1.7
Fluoride	10^{-2} M	542.6 ± 35.8

Tumour was chopped and homogenised at 4 °C with six strokes of a loose fitting Dounce homogeniser (10 ml per g wet weight 0.25 M sucrose, 1 mM EDTA in 10 mM Tris-HCl, *p*H 7.4). Centrifugation was carried out in 50-ml tubes in the SS-34 head of a Sorvall RC2B centrifuge. The centrifuge was switched on, allowed to reach 2,000 r.p.m., and immediately slowed down with the brake applied. The supernatant was centrifuged for 10 min at 4,300 r.p.m. The upper, creamy layer from this pellet was removed in the same buffer and centrifuged at 6,000 r.p.m. for 10 min. The pellet was resuspended in the adenylate cyclase assay buffer and immediately assayed (50 µl suspension per assay tube). Protein was measured by the method of Hartree[15]. Adenylate cyclase incubations were carried out at 37 °C for 12 min. Assay mixtures consisted of the following components in a volume of 100 µl: 25 mM Tris-HCl, *p*H 7.4; 1 mM α-^{32}P-ATP (specific activity about 10 c.p.m. pmol^{-1}, Radiochemical Centre); 1 mM cyclic AMP; 2.5 mM phosphoenol pyruvate; 1.5 IU pyruvate kinase; 0.013% (w/v) bovine serum albumin; 30 mM KCl; 4.5 mM $MgCl_2$; 160 µg membrane protein; and hormone concentrations as indicated. Reaction was stopped by addition of 100 µl 40 mM ATP and boiling for 2 min. The generated ^{32}P-cyclic AMP was purified by the method of Salomon *et al.*[16] as described previously[17]. Bovine PTH of potency 1,000 U per mg was used, and other hormones were as follows; salmon calcitonin (synthetic, gift of Armour Pharmaceuticals), prostaglandins E_1 and E_2 (gift of Dr J. E. Pike, Upjohn), and glucagon (Eli Lilly). In addition to the hormones indicated, the following had no significant effect on adenylate cyclase activity at the concentrations indicated: insulin (3×10^{-6} M); human growth hormone (3×10^{-6} M); ovine prolactin (3×10^{-6} M); pentagastrin (ICI, 10^{-5} M); arginine vasopressin (Sandoz, 10^{-5} M); human adrenocorticotropic hormone (6×10^{-6} M).

*Results are means ± s.e.m. of triplicate determinations.

human tumours[10–13] and in the premalignant stage of chemical carcinogenesis[4,14]. The significance of these changes is not yet clear, and no unified concept could be developed at present. The hormone-responsive transplantable osteogenic sarcoma described here will facilitate testing of the effect of hormone-mediated changes in cyclic AMP on malignant cell proliferation. Moreover, differentiation of the tumour will be particularly amenable to study in view of its osteogenic capability, allowing it to progress from a soft tumour to one which has a network of mineralised matrix; this lends itself to relatively simple quantification.

Supported by the Yorkshire Council of the Cancer Research Campaign and the Joseph Herman Trust (University of Melbourne). We thank Dr G. W. Tregear for synthetic PTH peptides.

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Received December 12, 1975; accepted January 30, 1976.

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Reduced muscle acetylcholine sensitivity in rats immunised with acetylcholine receptor

ANIMALS immunised with acetylcholine receptor (AChR) purified from the electric organ of *Electrophorus electricus* or *Torpedo marmorata* develop a muscular weakness which is alleviated by injections of anticholinesterases[1–6]. This observation has provided a valuable animal model for study of the disease myasthenia gravis. Sera from rats immunised with electric eel receptor contain antibody to AChR isolated from rat skeletal muscle[2,6], suggesting that the muscular weakness is due to a reduced endplate sensitivity to ACh. We report here a reduction in the ACh sensitivity of denervated muscles from immunised rats. Furthermore, there is a factor in the serum of immunised rats which can reduce the ACh sensitivity of denervated rat muscles *in vitro*.

Female Lewis rats (10 weeks old) were injected on a single occasion at multiple intradermal sites with 100 pmol of AChR protein, purified from the electric organ of *E. electricus*, in 0.1 ml of phosphate-buffered saline, emulsified in 0.1 ml of Freund's complete adjuvant. As additional adjuvant 2×10^{10} *Bacillus pertussis* organisms (special vaccine product, Eli Lilly) were injected subcutaneously. Rats immunised in this way develop two episodes of weakness; an initial transient episode after 8–9 d and a second chronic episode after 4 or more weeks.

Table 1 Reduction of extrajunctional ACh sensitivity of denervated hemidiaphragm muscle fibres by rat anti-AChR antiserum

Anti-rat ACh receptor titre (10^{-8} M)	Complement inactivation	Incubation period (min)	$\frac{\text{ACh sensitivity (serum treated)}}{\text{ACh sensitivity (control)}}$ (%)
1.03	No	120	23*
1.53	No	45	28*
1.31	No	75	29*
1.61	Yes	120	29†
0.62	Yes	60	43‡
0.24	Yes	75	50*

In some experiments complement was inactivated by heating the serum for 30 min at 56 °C. Extrajunctional ACh sensitivities were measured in 15–20 fibres of 1-cm wide muscle strips before and after incubation with sera for 45–120 min at 37 °C. All sensitivity measurements were made at 21–23 °C. Anti-rat AChR titre is expressed as mol ^{125}I-α-cobratoxin binding sites precipitated per l of serum[6]. This assay in which rat antibody bound to ^{125}I-α-toxin-AChR is precipitated by rabbit anti-rat IgG, measures antibody directed against sites on the AChR molecule other than the ACh binding site. Statistical significance was determined using a *t* test.

* $P<0.0005$.
† $P<0.025$.
‡ $P<0.05$.

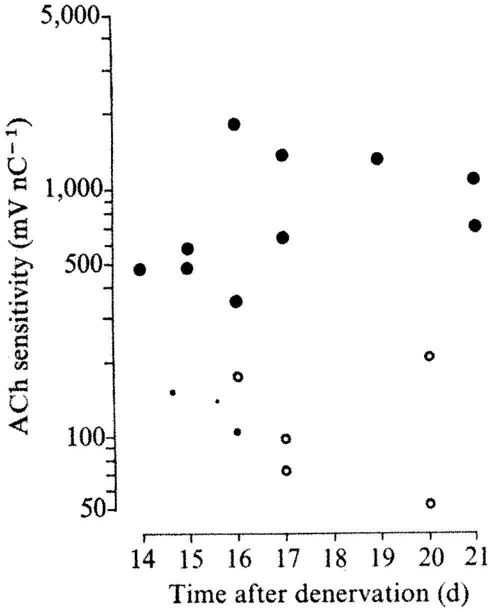

Fig. 1 Mean extrajunctional ACh sensitivities of denervated hemidiaphragm muscle fibres from adjuvant-treated rats (●) and rats immunised with AChR (○). Each point represents the mean sensitivity of 15–22 fibres measured at points 3–5 mm from the myotendonous junction. Extrajunctional ACh sensitivities were determined at room temperature (21–23 °C) by iontophoretic application of ACh from pipettes filled with 3 M AChCl (resistances 200–350 M Ω) positioned within 50 μm of the intracellular recording electrode. Sensitivities are expressed as mV nC^{-1} (ref. 10), that is the amplitude of the ACh potential in mV per electric charge in nC passed through the iontophoretic pipette.

All experiments were made on animals during the second episode shortly after the appearance of generalised weakness. Control animals were injected with adjuvants only. Rat sera were tested for antibodies to rat AChR by immunoprecipitation using ^{125}I-α-cobratoxin-labelled receptor as described elsewhere[6]. Isolated hemidiaphragm muscles were examined electrophysiologically at room temperature (21–23 °C) in a modified Krebs solution (150 mM NaCl; 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 11 mM glucose, 5 mM HEPES–NaOH, *p*H 7.4, bubbled with 100% oxygen) using standard techniques.

As reported previously[5], miniature endplate potential (m.e.p.p.) amplitudes were reduced in animals immunised with receptor. The mean m.e.p.p. amplitude at 20 control endplates was 0.74±0.06 mV (mean±s.e.m.) while m.e.p.p. amplitudes in muscles from immunised rats were usually less than 0.2 mV. The mean m.e.p.p. amplitude estimated at 34 endplates was 0.14±0.01 mV. This value, however, probably overestimates the true amplitude, as m.e.p.p. amplitude histograms were often highly skewed, suggesting that smaller m.e.p.p.s. were lost in the recording noise level. Several properties of muscle fibres which affect m.e.p.p. amplitudes were similar in control and immunised rats. Muscle fibre resting potential and the reversal potential of the endplate potential showed little or no change. Also input resistance and membrane time constant in fibres of immunised rats (0.39±0.02 MΩ (mean ±s.e.m., $n = 66$), 2.36±0.08 ms ($n = 25$)) were not significantly different (*t* test, $P > 0.1$) from the values in control animals (0.37±0.03 MΩ ($n = 57$), 2.22±0.06 ms ($n = 34$)). The decrease in m.e.p.p. amplitudes is therefore likely to result either from a reduction in the size of the ACh quanta released from the nerve terminals or from a reduced postjunctional response to ACh. The latter possibility was examined by measuring the ACh sensitivity of extrajunctional regions of denervated muscles. Although extrajunctional receptors differ in some respects to junctional AChRs[7], denervated muscle offers a convenient preparation for analysing changes in ACh sensitivity.

Hemidiaphragm muscle fibres were denervated under ether anaesthesia by sectioning the left phrenic nerve close to the muscle. Extrajunctional ACh sensitivities were determined after 2–3 weeks of denervation. The mean extrajunctional sensitivities of control denervated muscles ranged from 340–1,900 mV nC^{-1}, while muscles from immunised animals showed mean sensitivities of 51–200 mV nC^{-1} (Fig. 1). Resting potential, input resistance and membrane time constants were similar in muscles from control and immunised rats denervated for an equivalent period. The lower ACh sensitivity in immunised rats must therefore result either from a reduction in the number of functional AChRs in the muscle membrane or from a reduction in the amplitude or time course of the conductance change associated with individual receptor activation.

The reduction in ACh sensitivity in muscles of immunised rats could be due to an alteration in the function of receptors by the binding of circulating antibodies. To test this idea, the effect of immune sera on the function of ACh receptors was studied *in vitro*. Extrajunctional ACh sensitivities of denervated hemidiaphragm muscle fibres were measured before and after incubation for 45–120 min with either control rat sera or sera from immunised rats. Immune rat sera reduced extrajunctional ACh sensitivities (Table 1), while sera from control rats inoculated with adjuvants were without effect. This finding suggests that the lower extrajunctional ACh sensitivity of denervated muscles in immunised rats is due to receptor modification by circulating anti-receptor antibodies. Since the antigenic determinants on AChRs that react with anti-receptor antibodies are common to both junctional and extrajunctional receptors[9], the reduction of m.e.p.p. amplitudes in immunised animals may similarly be due to an antibody-mediated reduction in ACh sensitivity. This possibility is supported by the finding that sera from rabbits immunised with AChR can reduce m.e.p.p. amplitudes at frog motor endplates *in vitro*[5].

Further information about the mode of action of anti-receptor antibodies must await the results of experiments using ACh noise analysis. These will show whether antibodies act by inactivating receptors in an 'all-or-none' manner, or by modifying the conductance change associated with individual receptor activations.

We thank Carla Bevan and Janet Clark for technical assistance. This study was supported by grants from the Muscular Dystrophy Associations of America, the National Institutes of Health and the California Chapter of the Myasthenia Gravis Foundation. S.B. was supported by a fellowship from the Muscular Dystrophy Associations of America and S.H. by a National Institutes of Health research career development award.

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Received November 10, 1975; accepted January 9, 1976.

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Development of difference between red and white muscles in sensitivity to Ca^{2+} in the rabbit from embryo to adult

VARIOUS properties of the Ca^{2+}-activated troponin system are different in red (slow twitch) and white (fast twitch) muscle; these differences are in molecular weight[1,2], amino acid sequences of the subunits[3], Ca^{2+} binding to troponin[1,2], and biochemical measures of contraction (actomyosin ATPase activity and superprecipitation[4-7]). Although the preceding differences have been investigated, no report on physiological differences in Ca^{2+}-activated tension have been published. We compared the development of Ca^{2+}-activated tension in an oxidative muscle fibre (soleus) and that in a glycolytic muscle fibre (adductor magnus) of the rabbit from embryo to adult. We used a technique recently developed in our laboratory[5] for mechanically disrupting the sarcolemma of skeletal muscle fibres. We then examined the data, in view of the known biochemical differences, for possible clues to the roles that troponins have in the Ca^{2+} sensitivity of the various types of muscle.

The sarcolemmas of fibres (~1 mm long) from the soleus, (red), and adductor magnus, (white), muscles of rabbits were disrupted by homogenisation as originally described by Kerrick and Krasner[5]. One red fibre and one white fibre in identical solutions were studied simultaneously. The fibres were mounted into stainless-steel clamps in a tension transducer similar to that used by Hellam and Podolsky[6]. They were then immersed in relaxing and contracting solutions, and their steady-state tensions were recorded[7].

All solutions contained 70 mM of K^+, 10^{-7} M of H^+, 10^{-3} M of Mg^{2+}, 2×10^{-3} M of $MgATP^{2-}$, a total of 7×10^{-3} M of EGTA, and an ionic strength equal to 0.15. The major anions in the solutions were propionate and the *p*Ca of the test solutions was varied from 8 to 3.6. The relaxing solutions had a calculated *p*Ca of 8.0, and the maximally contracting solution had a *p*Ca of 3.6. Imidazole propionate was varied in all solutions such that ionic strength was held at 0.15. The total concentrations of K propionate, Mg propionate, Ca propionate, Na_2ATP, Na_2CP, and imidazole needed for the desired concentrations of the various ionic species and the ionic strength were determined by computer program[8]. The binding constants used in the program were taken from the literature[8].

Data points for each of the *p*Cas were determined and the means and standard errors of the means computed. In general, one data point for each *p*Ca was collected for each fibre and several fibres were used. When a fibre broke or generated too small a force, a new fibre was mounted and studied.

The sensitivity of red muscle to Ca^{2+} was approximately 2.5 times greater than that of white muscle in the adult (Fig. 1*a*). In contrast, Ebashi *et al.* found that the calcium binding to troponin in white muscle was greater than in red. Both red and white muscle showed the same sensitivity to calcium ion in the embryo (Fig. 1*c*), but red muscle had about ten times greater sensitivity to $[Sr^{2+}]$ than white muscle in the adult (Fig. 1*d*). Sensitivity to calcium ion increases in red muscle with development from 26-d embryo to adult (Fig. 1*b*), but remains constant in white muscle (Fig. 1*c*). The difference in sensitivity between red muscle and white muscle develops from embryo to adult, primarily from increasing sensitivity to Ca^{2+} or Sr^{2+} (Fig. 1*d*) in the red muscle.

These data show that sensitivity to calcium ion and strontium ion changes during development in the soleus muscle but not in the adductor magnus muscle. Thus, if troponin is entirely responsible for muscle sensitivity to calcium ion, then changes in the troponin molecule itself must take place during development.

In contrast, studies of the biochemical measures of contraction have shown greater Ca^{2+} sensitivity of Ca^{2+}-activated ATPase activity and Ca^{2+} binding to troponin in white muscle than in red[7]. Thus, these differences are not in agreement with the differences in the biochemical measures of contraction[7]. The discrepancy between the two may be due to either disruption and isolation or removal of proteins, such as light chains of myosin.

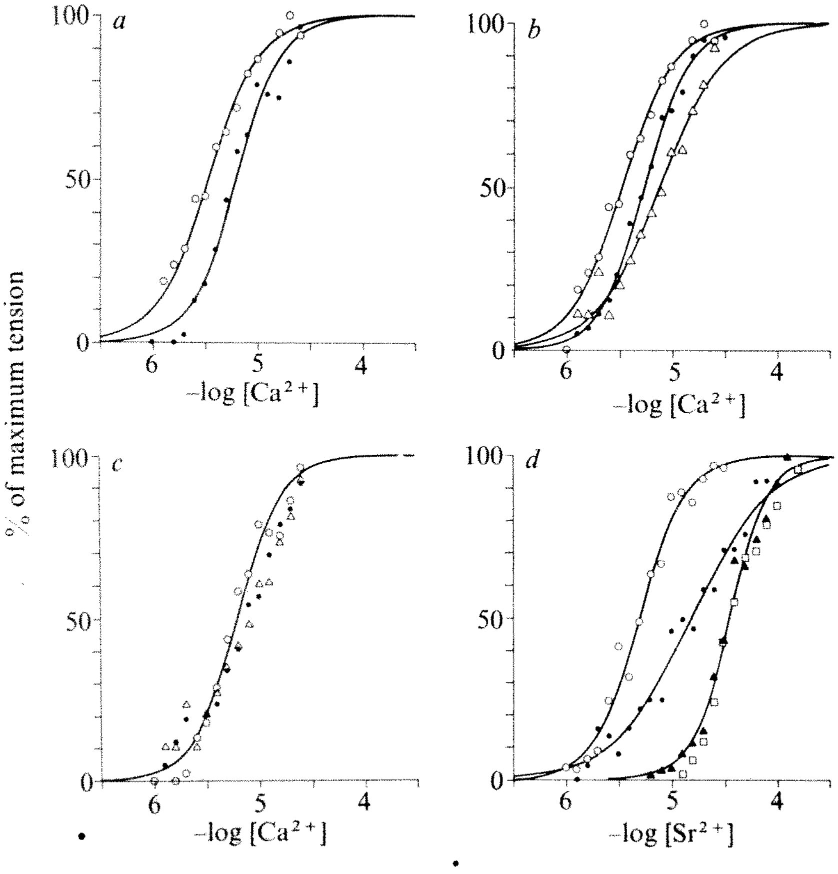

Fig. 1 The relationship between *p*Ca or *p*Sr ($-\log_{10}(X^{2+})$) (M) and percentage of maximum tension for mechanically disrupted rabbit adductor and soleus muscle fibres during development. Smooth curves were obtained by fitting the data to the Hill equation

$$\log_{10}\left(\frac{\%T}{100-\%T}\right)=n\log_{10}(X^{2+})+R$$

where *n* and *R* are constants. *a*, *p*Ca–tension relationship for adult rabbit soleus (○) ($n = 1.7$, $R = 9.6$) and adductor (●) ($n = 2.0$ and $R = 10.5$). *b*, *p*Ca–tension relationship for rabbit adult soleus (○) ($n = 1.7$, $R = 9.6$), 5-d-old soleus (●) ($n = 2.0$, $R = 10.5$), and embryo soleus (△) ($n = 1.4$, $R = 7.3$). *c*, *p*Ca–tension for rabbit adult adductor (○) ($n = 2.0$, $R = 10.5$), 26-d embryo adductor (●), and 26-d embryo soleus (△). *d*, *p*Sr–tension for rabbit adult soleus (○) ($n = 2.0$, $R = 10.5$) and adductor (□) and 5-d-old soleus (●) ($n = 1.2$, $R = 5.7$) and adductor (▲) ($n = 2.3$, $R = 10.2$). The largest s.e.m. computed for any data point was ±5.5%.

Unfortunately, no data have been published on the biochemical changes that occur in the troponin of the rabbit's red muscle and that of white muscle during development. Biochemical differences in subunits of troponin between red and white muscle have, however, been reported. One of these subunits (TNI) from white muscle inhibits Mg^{2+}-activated actomyosin ATPase activity of desensitised actomyosin more effectively than TNI from either red muscle or cardiac muscle. Studies of subunits from skeletal and heart muscles have also indicated differences in effect on actomyosin ATPase[9]. Because in the above studies researchers used incomplete contractile protein systems or preparations of more than one type of muscle, their experimental results cannot be related directly to the results reported in this paper.

Fluorescent antibody techniques show that three types of troponin and heavy chains of myosin (red slow, white fast, and cardiac) exist in individual chick muscle cells and that during development to adult only the troponin and myosin that are characteristic of a muscle type are retained[10]. One possible interpretation of these findings is that the troponin characteristic of white muscle may dominate the Ca^{2+} control of embryonic muscle, but is superseded during development by the troponin characteristic of red or cardiac muscle.

There is some evidence of a Ca^{2+}-sensitive regulatory system in the myosin molecule. It has been clearly demonstrated in scallops[11]. Haselgrove[12] and Huxley[13], using frog skeletal muscle that had been stretched so that there was no overlap of the actin and myosin filaments, demonstrated that the X-ray diffraction pattern associated with the myosin filaments changed when the muscles were electrically activated. Chaplain and Gergs[14], using X-ray diffraction, noted that the cross bridges moved away from the thick filament on the addition of Ca^{2+} in adult glycerinated rabbit muscles stretched so that there was no overlap of thick and thin filaments. Further evidence for a conformational change of myosin comes from Morimoto and Harrington[15]. Using rabbit back muscles, they measured sedimentation coefficient and found it to change in a range from 10^{-6} to 10^{-5} M Ca^{2+} concentration; they then noted that the viscosity of myosin changed in this range of concentrations.

The light chains of myosin may modulate Ca^{2+} control of muscle since Ca^{2+} binding has been associated with the DTNB light chains of myosin in the rabbit[14]. The light chains of white muscle in the chick embryo are reported to be more similar to the light chains of white muscle in the adult than the red; this finding is consistent with our finding of unchanged Ca^{2+} sensitivity of white (adductor) muscle during development[16]. The myosin from white muscles show characteristic light chain patterns that are different from those of red muscles of the rabbit[16]; possibly, the development of light chains in red (soleus) muscle may have some role in the difference in and the change in development of Ca^{2+} sensitivity observed in this study.

We conclude that Ca^{2+} binding to troponin in red and white muscles does not entirely determine the sensitivity of these muscles to Ca^{2+}, in spite of indications by previous biochemical experiments; that differences either in the troponin molecule or in light chains of myosin, or other unknown factors are responsible for the difference in sensitivity of calcium between the two types of muscles; and that molecular changes from embryo to adult change the Ca^{2+} sensitivity in red muscle. These data show the importance of using muscles of one type in experiments designed to show the exact molecular mechanism for activation of a contraction by Ca^{2+}.

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Is chromatid-type damage in ataxia telangiectasia after irradiation at G_0 a consequence of defective repair?

THE radiosensitivity of patients with ataxia telangiectasia (AT) has been indicated by *in vivo* studies[1–3] and *in vitro* studies on skin fibroblasts[4]. We present data here to show that this radiosensitivity is observed at the chromosomal level in blood lymphocytes X irradiated at different stages of the cell cycle and for the same range of doses as our survival experiments[4]. Our results are consistent with the hypothesis that there is a defect in some form of DNA repair in these patients. From the observation of the types of chromosome aberrations seen in AT lymphocytes after irradiation at G_0/early G_1, we propose a mechanism for the origin of these aberrations, based on the premise that the G_0/early G_1 chromosome contains a single DNA double helix, in which one polynucleotide chain can be damaged independently of the other.

From previous work on radiation cytogenetics we expected to see only chromosome-type damage (rings, dicentrics and fragments) in the mitosis following G_0 irradiation[5]. These observations have led to the concept that the chromosome at this phase responds to X irradiation as though it were a single linear entity[6]. Chromatid-type damage according to this theory is seen after irradiation in late G_1, S and G_2.

Heparinised blood was obtained from four AT patients and five controls. Table 1 shows the results of full chromosome analyses of unirradiated lymphocytes. Spontaneous chromosome aberrations are a recognised feature of AT[7]. Although gaps and breaks are fewer in these four patients than in others previously reported[7], the number of cells with non-clonal rearrangements is greater in AT patients than in normals. (Two AT patients show the presence of a lymphocyte clone.) Irradiated lymphocytes were analysed for the presence of chromosome- and chromatid-type aberrations. The results of irradiation with 200 and 400 rad in G_0 are shown in Table 2. AT lymphocytes do not show rings and dicentrics at a consistently higher frequency than controls, but the total number of fragments (terminal and interstitial deletions) is greater in AT cells. It is accepted that one fragment is associated with each major exchange[8], but allowing for incomplete exchanges, the ratio of fragments to rings and dicentrics is expected to be greater than unity. In our controls this ratio was 1.59 after each dose of radiation. The ratio was higher in AT cells and increased with larger X-ray doses (1.77 at 200 rad and 2.44 at 400 rad). In AT cells therefore there is a large excess of deletions which are not associated with chromosome-type exchanges.

Our results also show that AT cells exhibit chromatid-type damage when irradiated in G_0. This is seen as a large number of gaps and breaks, but most striking is the appearance of numerous chromatid interchanges, particularly triradials (Fig. 1*a*). The presence of these interchanges at

Table 1 Chromosome analysis of lymphocytes from AT and controls

Patients	Sex	No. of cells analysed	Total no. of rings and dicentrics	Total no. of fragments	Total no. of gaps and breaks	No. of cells with stable changes	Total no. of abnormal cells	Clone present
AT								
AT 4BI	M	100	0	0	17	6	19	–
AT 5BI	M	100	24	2	5	18	38	+
AT 6BI	F	100	2	3	3	16	24	+
AT 7BI	F	100	1	2	11	5	17	–
Controls								
CON 1BI	M	100	1	0	14	2	15	–
CON 2BI	F	100	0	1	8	1	10	–
CON 3BI	M	100	0	2	5	1	8	–
CON 4BI	F	100	0	0	8	1	8	–

0.4 ml blood was cultured in 4.0 ml Ham's F10 medium supplemented with 10% bovine serum, 1% phytohaemagglutinin, penicillin and streptomycin (100 IU ml^{-1}). Unirradiated lymphocytes were fixed after 48 h in culture, during their first division.

a high frequency after G_0 irradiation clearly distinguishes AT cells from controls. Chromatid-type damage of any kind is rarely seen in normal lymphocytes irradiated at G_0.

Irradiation during G_2 induced a very high level of chromatid gaps and breaks in AT lymphocytes (Fig. 1*b*) compared with controls (Table 3), agreeing with a previous report[9]. The frequency of chromatid interchanges was again higher in AT cells after both radiation doses, although interchanges were also present in irradiated controls at G_2. So far we have no data on subchromatid aberrations which can be observed at the second mitosis after irradiation at G_2.

We investigated the possibility that some AT lymphocytes showing triradial formation after irradiation at the time of culture initiation, were in G_2 and not in G_0. By scanning lymphocytes from uncultured blood on a Vickers microdensitometer, we found that the DNA content in all cells (from two AT patients and two controls) was consistent with their being in G_0. Cells in G_2 were used as positive controls. This evidence, together with the fact that unstimulated lymphocytes cultured for 48 h showed no mitoses, suggests that all lymphocytes from the AT patients were in G_0 at the time of irradiation. We believe, therefore, our observations show that chromatid damage by X rays is not restricted to late G_1, S or G_2 in AT cells, but can occur after irradiation at G_0.

To explain the types of chromosome aberration seen in both AT and normal cells after irradiation at G_0 we propose that the chromosome at G_0 and in early G_1 behaves as a double-stranded structure. While we appreciate the difficulties of extrapolating from observations on chromosomes to the molecular level, we suggest that the observations are consistent with the basic unit being the DNA double helix. X rays can resolve the two strands at G_0, producing single-strand damage (breaks or base damage). In normal cells such damage is usually repaired before S, since chromatid aberrations are rarely seen at mitosis. The chromatid-type damage after late G_1 irradiation may be a consequence of insufficient time between G_1 and S phase for single-strand damage to be repaired (as Bender suggested[11]). In AT cells, however, we suggest that much of the damage to single strands of DNA is not repaired and persists to manifest itself as chromatid-type damage (including triradials) at the next mitosis. Double-strand damage produces chromosome-type aberrations after G_0

Table 2 Chromosome analysis after X irradiation in G_0

Patients	Ring	Dic.	Frag.	tg	tb	cg	cb	Tric.	Tid. frag.	Chromatid interchanges Trirad.	Quadri.	Other
200 rad												
AT												
AT 4BI	11	29	103	11	5	4	0	0	0	4	0	0
AT 5BI	14	44	79	11	6	2	1	0	1	7	1	0
AT 6BI	6	29	62	6	3	5	2	0	2	0	1	0
AT 7BI	9	32	64	14	1	0	0	1	0	2	1	0
Controls												
CON 1BI	6	25	34	11	0	3	0	0	0	0	0	0
CON 2BI	ND											
CON 3BI	5	22	47	8	1	0	0	0	0	1	0	0
CON 4BI	0	29	61	6	0	1	0	0	0	0	0	0
400 rad												
AT												
AT 4BI	18	75	264	21	18	3	1	5	0	12	1	2
AT 5BI	19	79	217	38	20	7	2	3	6	8	0	2
AT 6BI*	10	37	75	6	4	0	0	1	5	0	0	0
AT 7BI	20	66	235	32	19	2	0	0	0	14	2	4
Control												
CON 1BI	18	77	145	8	1	0	0	8	0	1	0	0
CON 2BI	10	95	167	4	2	3	0	3	1	1	0	0
CON 3BI	10	90	170	2	1	2	0	1	0	0	0	0
CON 4BI	14	78	142	3	1	2	1	1	4	0	0	0

ND, Not done; Ring, rings; Dic., dicentrics; Frag, fragments; tg, chromatid gaps; tb, chromatid breaks; cg, chromosome gaps; cb, chromosome breaks; Tric., tricentrics; Tid. frag, chromatid fragments; Trirad., triradials; Quadri., quadriradials.

*Only 22 cells analysed.

X irradiation was carried out using a Pantak X-ray machine (300 keV, 12 mA, half value thickness 1.5 mm Cu, FSD 50 cm, 180 rad min^{-1}). Whole blood was irradiated at G_0, that is, immediately before being put into culture. Doses of 200 and 400 rad were used. The lymphocytes were collected in their first post-irradiation division at 52 h and 50 cells were analysed for each dose used.

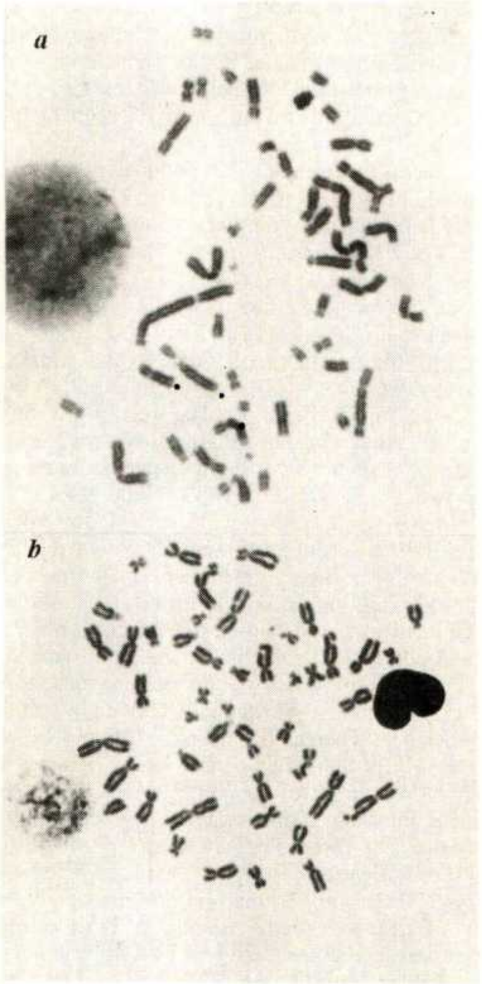

Fig. 1 *a*, Triradial chromosome (chromatid-type damage) together with dicentrics (chromosome-type damage) in an AT lymphocyte irradiated with 400 rad at G_0. *b*, Level of chromatid damage (gaps and breaks) in an AT lymphocyte irradiated with 200 rad at G_2.

irradiation, and chromatid damage after G_2 in normals. The high level of deletions after G_0 irradiation and chromatid-type damage after G_2 in AT cells, implies either a high level of double-strand breakage or failure to rejoin. Using alkaline and neutral sucrose gradients, however, we have previously shown normal single-strand rejoining and also, we believe, normal double-strand rejoining in AT cells[4]. The enhanced levels of both types of chromosome aberrations in AT cells after normally sublethal doses of X irradiation may be a consequence of a failure to excise lesions in the DNA. Paterson *et al.*[10] have confirmed that AT cells show normal single-strand rejoining and, using supralethal doses of γ rays, have shown that AT cells are defective in excision repair of γ-ray induced base damage.

Many reports[11–16] have speculated on the possibility that incomplete repair mechanisms facilitate resolution of molecular damage at the chromosomal level, including aberrations induced by ultraviolet light and chemical compounds. The possible role of failure to repair damage in the production of chromosome aberrations has been shown in cells from patients with xeroderma pigmentosum (XP)[17]. The high incidence of malignancies in both AT and XP may be a consequence of the defective DNA repair in both of these syndromes, giving rise to cytogenetically abnormal cells with a potential to become malignant.

We thank Professor J. H. Edwards, Drs S. Bundey, W. Schutt, R. A. Thompson and M. M. Oo for the cases of AT; Dr P. Fowles for use of facilities in the Birmingham Radiation Centre; Dr H. Wallace for the use of the microdensitometer, and Miss C. J. Harrison, Mrs K. E. Vause and Mr P. Wattie for technical assistance. This work was supported by the Cancer Research Campaign and the Medical Research Council.

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Received December 22, 1975; accepted January 28, 1976.

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Table 3 Chromosome analysis after X irradiation in G_2

Patients	Ring	Dic.	Frag.	tg	tb	cg	cb	Tric.	Tid. frag.	Chromatid interchanges		
										Trirad.	Quadri.	Other
100 rad												
AT												
AT 5BI	0	10	12	93	44	2	0	0	4	5	2	0
AT 6BI	0	1	5	113	41	0	0	0	11	3	3	0
Controls												
CON 2BI	0	1	2	14	12	0	0	0	0	0	4	0
CON 5BI	0	0	1	14	3	1	0	0	0	0	1	0
200 rad												
AT												
AT 5BI	0	9	26	146	102	4	3	1	6	14	2	3
AT 6BI*	0	1	6	119	61	2	0	0	3	1	7	0
Controls												
CON 2BI	0	1	4	15	10	1	0	0	2	0	1	1
CON 5BI	0	0	2	18	9	3	0	0	0	3	1	0

*Only 25 cells analysed.
Lymphocytes in G_2 were irradiated for 4 h before collecting at 52 h. 50 cells were analysed per person for each dose.

Defective excision repair of γ-ray-damaged DNA in human (ataxia telangiectasia) fibroblasts

ATAXIA telangiectasia (AT) (Louis–Bar syndrome) is a rare human neurovascular disease displaying an autosomal recessive pattern of inheritance[1-3]. Affected individuals, although clinically normal at birth, symptomatically develop cerebellar ataxia (loss of muscular coordination) and oculocutaneous telangiectasis (chronic dilation of the small blood vessels) in early childhood. The course of the disease follows a variable progression commonly leading to total neurological incapacitation before puberty. Accessory complications include lymphoreticular neoplasia[2,3], bronchiectasis[1,2], recurrent sinopulmonary infections[1,2], decreased levels of serum immunoglobulins IgA and IgE (refs 3 and 4), impaired cellular immunity[3,4], and widespread chromosomal instability[5]. AT patients, on receiving conventional radiotherapy for tumour treatment, tend to develop unusually severe complications often culminating in premature death[6-8]. Pronounced radiosenitivity is also observed at the cellular level in laboratory studies; the number of radiation-induced chromosomal aberrations is enhanced in leukocytes obtained from AT donors[9]. Moreover, diploid fibroblasts cultured from affected individuals exhibit a reduced ability to form colonies following exposure to γ radiation[10] and radiomimetic chemicals[11]. Since the principal damage induced by both types of agents occurs in the DNA and seems to be acted on by the same enzymatic repair mechanisms[12,13], it would seem probable that the molecular basis for the clinical radiosensitivity of AT patients stems from a deficient DNA repair mechanism. We therefore measured the DNA repair properties of AT fibroblasts after exposure to γ radiation. Data presented below provide direct biochemical evidence that diploid strains from AT donors are indeed impaired in DNA repair; in particular, these cell lines possess an enzymatic defect in an excision-type repair process operating on γ-modified nitrogenous base residues.

The ability of AT strains to carry out two repair processes, rejoining of γ-induced single-strand breaks (that is, scissions in the sugar–phosphate backbone of individual polynucleotide chains) and excision of γ-modified base residues, was evaluated with the aid of two complementary physicochemical techniques: (1) *in vitro* enzymatic assay[14,15] and (2) equilibrium density-gradient centrifugation[16]. The enzymatic assay monitors the DNA separately for the restitution of strand breaks and for the disappearance of base defects, during *in vivo* incubation after γ irradiation. Base defects are detected as sites susceptible to γ endonuclease activity in a crude extract of the bacterium *Micrococcus luteus*[17] (referred to as γ endonuclease-susceptible sites). The equilibrium centrifugation technique measures the magnitude of radiation-induced repair replication; this parameter reflects the sum total of nucleotides inserted at damaged sites along each strand during the course of repairing both types of radioproducts[18].

Our experiments were carried out with five (two control, three AT) human diploid strains: CRL 1141 and CRL 1147, cultured from two clinically normal volunteers; and AT3BI, CRL 1312, and CRL 1343, derived from three AT patients of unrelated kindred (for details, see Table 1).

To monitor the metabolic fate of γ radioproducts, irradiated cultures of normal (CRL 1141) and AT (AT3BI) strains were incubated and the numbers of single-strand breaks and γ endonuclease-susceptible sites remaining in the DNA at various times were measured (Fig. 1). The restitution of directly induced strand breaks in AT cells occurred with exponential kinetics indistinguishable from those displayed by normal cells. The other two AT strains, CRL 1312 and CRL 1343, also exhibited similar strand-rejoining kinetics (data not shown). Thus, in spite of their pronounced sensitivity to γ inactivation[10], AT strains seem to possess a normal enzymatic mechanism for rejoining radiation-induced single-strand breaks. Others[10,20] have independently arrived at the same conclusion.

In marked contrast to the efficient repair of strand breaks, the rate of disapperance of γ endonuclease-susceptible sites was more than four times slower in AT3BI than in CRL 1141 cells. For instance, whereas the normal strain achieved 80% site removal within 3 h, the mutant strain required twice as long to act on only 50% of the initial sites. In as much as (1) the strand-rejoining mechanism seems to proceed in a manner similar to the last three steps in excision repair (excision, repair synthesis, ligation), probably using similar, if not the same, enzymes[12,13]; and (2) this rejoining process seems to be normal in AT strains, it seems reasonable that the time course of site disappearance reflects the first step in excision repair of γ-modified base residues *in vivo*. Thus, the simplest conclusion is that the AT3BI strain lacks a fully functional γ endonuclease considered to initiate this excision-repair process.

If the observed disappearance of γ endonuclease-susceptible sites indeed reflects an excision-repair process acting on γ-induced base damage, it follows that the impaired ability of AT3BI cells to effect site removal should be accompanied by a reduction in the magnitude of γ-stimulated repair replication. That is, a defective capacity to excise base defects should accordingly lead to a decrease in the number of gaps which need to be filled by repair synthesis. We therefore measured the amount of repair replication occurring in normal and AT strains during 2 h after irradiation (Fig. 2). The level of repair replication detected in two normal cell lines, CRL 1141 and

Table 1 General description and *in vitro* characteristics of diploid fibroblast strains from normal and AT donors*

Strain designation	Clinical description	Donor Age (yr)	Donor Sex	*In vitro* age during assay†	Doubling time (h)
CRL 1141 (Ba Del)	Normal	3	M	18–28	38–43
CRL 1147 (Ter Loy)	Normal	9	F	10–19	41–49
AT3BI	AT	4	M	26–34	39–44
CRL 1312 (Sa Gru)	AT	7	F	9–17	42–47
CRL 1343 (Se Pan)	AT	7	F	10–21	36–40

*Tissue origin: human skin.

†Expressed as cell population doublings since biopsy, although cultures were actually diluted 1:3 at each subcultivation.

AT3BI was provided by A. R. Lehmann, University of Sussex; the remaining four were obtained from the American Type Culture Collection, Rockville, Maryland. Using standard cell culture procedures, monolayer stocks of each fibroblast strain were routinely maintained at 37 °C in thymidine-free F12 medium[19] supplemented with 15% (v/v) foetal calf serum (Gibco) and a penicillin–streptomycin mixture (100 IU each $\mathrm{ml^{-1}}$). Unless otherwise indicated, all cell culture supplies were purchased from Microbiological Associates Inc.

Experimental cultures in late logarithmic growth phase were prepared by seeding $\approx 8 \times 10^5$ cells in plastic Petri dishes (15 cm diam., Lux Scientific) followed by incubation for 24–48 h at 37 °C. In this and subsequent experimental incubations the F12 medium contained newborn (10%, v/v) rather than foetal calf serum. Radiation treatment was administered at ice-bath temperature with either *a*, ^{60}Co γ rays (dose rate, 26–27 krad $\mathrm{min^{-1}}$) delivered in a Gammacell 220 (Atomic Energy of Canada Ltd) or, in certain control studies, *b*, far ultraviolet radiation (chiefly 254-nm wavelength; incident fluence rate, 1.1 $\mathrm{J\,m^{-2}\,s^{-1}}$)[14]. Cultures were maintained under anoxia during γ irradiation by introducing a stream of N_2 gas into aspirated Petri dishes for 5 min before, and during the exposure. All remaining experimental manipulations and treatment schedules, including those involved in the *in vitro* enzymatic assay and equilibrium density-gradient centrifugation, were performed as described briefly in the figure captions and in detail elsewhere[14-16].

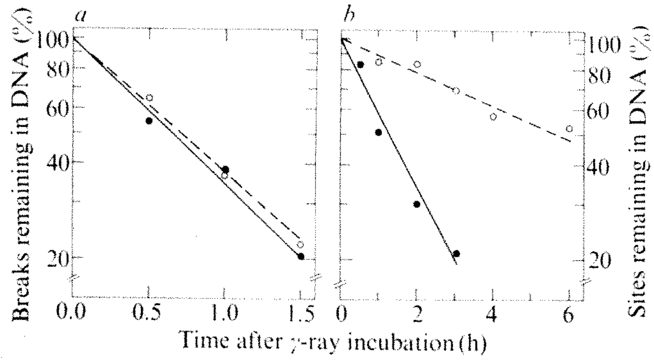

Fig. 1 Time-dependent disappearance of γ radioproducts from the DNA of normal (●, CRL 1141) and AT (○, AT3BI) fibroblasts exposed under anoxia to 50 krad ^{60}Co γ-radiation. *a*, Single-strand breaks and *b*, γ endonuclease-susceptible sites were monitored by an *in vitro* enzymatic assay similar to that described earlier for the quantitation of ultraviolet-induced pyrimidine dimers[14,15]. The DNA of the cells was labelled for 24–48 h with either ^{3}H-methylthymidine (specific activity, 2 Ci mmol^{-1}) or 2-^{14}C-thymidine (specific activity, 45–53 mCi mmol^{-1}) (Amersham, Searle) added to the F12 incubation medium at a concentration of 1.0 μCi ml^{-1}. After removing the radioactive medium, ^{3}H-labelled cultures of each strain were irradiated and immediately incubated for various times in parallel with ^{14}C-labelled, unirradiated (control) cultures. Parallel cultures were lysed and their ^{3}H- and ^{14}C-DNAs were coextracted *in toto*. Two samples of the purified DNA were then incubated for 30 min at 37 °C, one with, and the other without, a crude cell-free extract from *M. luteus* (the equivalent of fraction II of Carrier and Setlow[25]). Typically, the 200-μl reaction solution contained: 2 μmol potassium phosphate (*p*H 7.5); 0.2 μmol EDTA; 0.2 μmol 2-mercaptoethanol; 1 μg ^{3}H-labelled, irradiated DNA (≈15,000 d.p.m.); 1 μg ^{14}C-labelled, unirradiated DNA (5,000 d.p.m.); 10 μg tRNA (General Biochemicals); 20 μg (protein) *M. luteus* extract (an amount in excess of that needed to complete the endonuclease reaction). At the completion of incubation 50 μl 2 N NaOH was added, and the samples were subjected to velocity sedimentation (40,000 r.p.m. at 20 °C for 135 min in a Spinco SW-56 rotor) in alkaline sucrose gradients (5–20%, w/v; 0.5 M NaCl; 0.21 N NaOH; 10 mM EDTA). After fractionation and liquid scintillation counting, computer analysis of the radioactivity distributions in the gradient profiles yielded the number of single-strand breaks in the irradiated, compared with non-irradiated, DNA. The value obtained for the DNA sample incubated alone is taken as the number of directly induced strand breaks remaining in the radiation-damaged DNA, whereas the additional breaks in the extract-treated sample represent the number of nuclease-susceptible sites. Data for each curve were normalised by expressing the lesion incidence in DNA from incubated cultures as a percentage of the corresponding value for the non-incubated culture, that is, the number initially produced per 10^{7} daltons DNA = 100% (≈0.8 single-strand breaks; ≈1.2 nuclease-susceptible sites). Experimental points represent the average of triplicate determinations (s. d. ≤ 13%).

CRL 1147, increased rapidly with γ-ray doses up to 75 krad at which stage the level seemed to approach a plateau value. Although the general response of three AT strains, including AT3BI, to radiation treatment was also dose dependent, the maximal extent of repair replication attained by each (45–60 d.p.m. per μg DNA) was only about half that (≈110 d.p.m. per μg DNA) found in the normal strains. It seems, therefore, that the level of repair replication attained in the AT strains after γ irradiation is lower than that achieved by the normal strains, providing additional evidence that AT strains are impaired in excision repair of γ-induced base defects.

It was crucial to establish further that the observed malfunction was specific and not an artefactual consequence of some systematic peculiarity in the general properties of the mutant strains; particularly as excision repair of γ radioproducts was not totally absent from the AT strains. Repair efficiency was independent of biological age, both *in vivo* (chronological age of donor at biopsy) and *in vitro* (age in culture). The repair deficiency in AT strains did not simply stem from a general depression in cell growth rate in culture. The population doubling times for the two normal and three mutant cell lines did not vary greatly, nor was a positive correlation found between the kinetics of excision repair and growth rate (see Table 1). All strains were mycoplasma-free as judged by assaying exogenous ^{3}H-uracil/^{3}H-uridine incorporation into RNA[21].

The response of AT strains to ultraviolet radiation was also monitored, since excision repair of ultraviolet photoproducts and γ radioproducts is thought to be mediated by parallel mechanisms[13] which, except for specific endonucleases acting on the different ultraviolet and γ lesions[12,13], may well use common enzymes. As is evident in Fig. 3, normal CRL 1141 and mutant AT3BI cells executed excision repair of ultraviolet photoproducts with identical proficiencies, assayed as either the rate of removal of ultraviolet endonuclease-susceptible sites (that is, pyrimidine dimers[14]) or the level of ultraviolet-stimulated repair replication. The other two mutant strains, CRL 1312

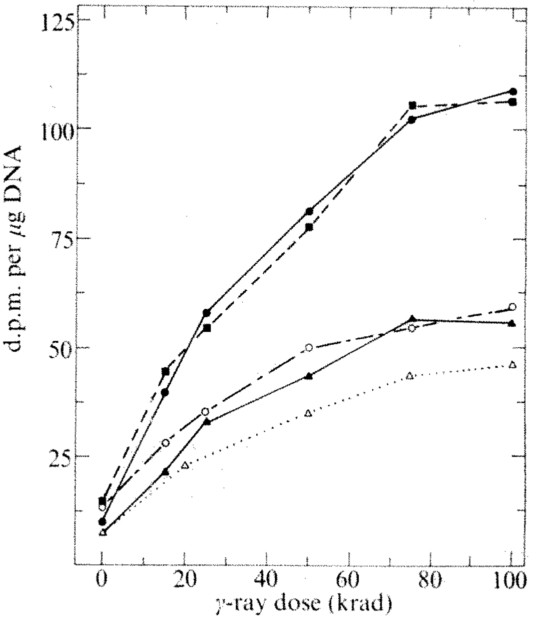

Fig. 2 Dose-dependent levels of repair replication induced in the DNA of normal (●, CRL 1141; ■, CRL 1147) and AT (○, AT3BI; ▲, CRL 1343; △, CRL 1312) strains by ^{60}Co γ-radiation delivered under anoxia. To assess their ability to carry out γ-stimulated repair replication, the human cell lines underwent a standard regimen designed to maximise the incorporation of exogenous nucleotides into DNA at repaired sites while inhibiting *de novo* synthesis[18]. Unlabelled cultures containing 2–4 × 10^{6} fibroblasts were grown for 2 h in F12 medium containing 65 μM bromodeoxyuridine (BUdR) and 1 μM fluorodeoxyuridine (FUdR), γ-irradiated under nitrogen, and immediately labelled for 2 h with 10 μCi ml^{-1} ^{3}H-methylthymidine (specific activity, 45–53 Ci mmol^{-1}; Amersham, Searle) in F12 medium supplemented with 65 μM BUdR, 1 μM FUdR and 1 mM hydroxyurea. Finally, the cultures were incubated for an additional 1 h in F12 medium containing 65 μM BUdR and 1 μM FUdR. The extent of repair replication occurring during the postradiation labelling period was determined by equilibrium centrifugation of the radioactive and density-labelled DNA in NaI gradients. The method followed was basically that developed by Lohman *et al.*[16] except that one rebanding of 'light' parental DNA was normally necessary to ensure its complete physical separation from contaminating 'heavy' DNA arising from semiconservative *de novo* synthesis. After lysis and DNA extraction, neutral NaI gradients (6 ml, containing 0.22 mmol Na_2SO_3, 10–20 μg extracted human DNA, 0.1 mg ethidium bromide, 4.69 g NaI, adjusted with addition of either Na_2SO_3 or NaI to 1.5400 g cm^{-3} final density) were prepared and centrifuged to equilibrium (33,000 r.p.m. at 20 °C for at least 60 h in a Spinco Type 40 rotor). After collection (~20 fractions per gradient) the light DNA was pooled (normally six 300-μl fractions) and dialysed overnight against a 10 mM Tris–0.15 M NaCl (*p*H 7.5)–10 mM EDTA buffer. NaI gradients containing the dialysed DNA were prepared and centrifuged as above. After fractionation, the distributions of DNA (measured fluorometrically) and radioactivity were ascertained and analysed to calculate the magnitude of repair replication, expressed as d.p.m. per μg light DNA (typically comprising five 300-μl peak fractions). Datum points represent the average of five or more determinations (s.d. ≤ 15%).

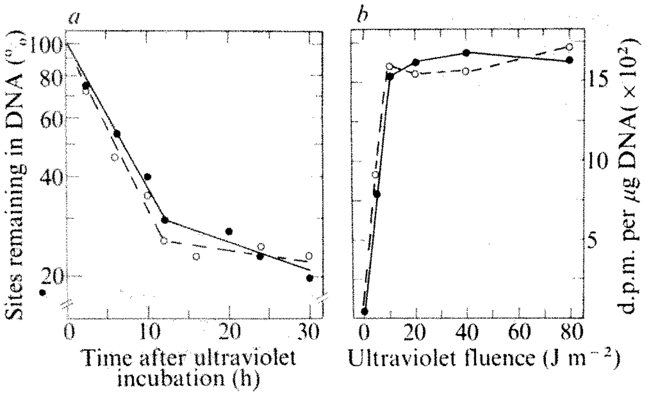

Fig. 3 Excision repair of ultraviolet photoproducts in the DNA of normal (●, CRL 1141) and AT (○, AT3BI) fibroblasts as reflected by *a*, time course of disappearance of ultraviolet endonuclease-susceptible sites; *b*, magnitude of ultraviolet-stimulated repair replication. The fate of nuclease-susceptible sites was monitored with the *in vitro* enzymatic assay as outlined in the caption of Fig. 1 except that: (1) ^{3}H-labelled cultures were exposed to 5 J m^{-2} 254-nm radiation, and (2) extracted DNA was incubated with highly purified *M. luteus* ultraviolet endonuclease (10 μl per DNA–protein reaction mixture) selectively active toward pyrimidine dimers in ultraviolet-irradiated DNA[14]. (The ultraviolet endonuclease was a gift of H. L. Heijneker, Leiden State University, Leiden, The Netherlands.) The amount of repair replication occurring in cultures during 2 h after exposure to specified ultraviolet fluences was determined essentially as described in Fig. 2 except that accurate measurements were obtained without the necessity of rebanding light parental DNA. 100% sites = 2.7 per 10^7 daltons (*a*). Datum points represent the average of triplicate determinations (s.d. $\leq 10\%$).

and CRL 1343, also carried out ultraviolet-induced excision repair with normal proficiency (data to be published elsewhere). It therefore seems that the enzymatic defect in the AT cell lines specifically affects the endonuclease active on γ-modified base residues.

Although the resolution of our assays compelled us to trace the fate of γ radioproducts in moribund cell populations, we are reasonably confident that, within the short postradiation intervals considered here, the metabolic responses assayed portray the actions of *bona fide* repair processes since: (1) the cultures seemed to be normal, both at the cellular and subcellular levels; for example, both control and AT strains remained in monolayer with few floating cells and their nuclear DNA was not subject to rampant degradation; (2) control strains, in particular, seemed to achieve considerable success in restoring their DNA to an undamaged stage (Fig. 1).

As observed in *Escherichia coli* minicells[17], the ratio of γ endonuclease-susceptible sites to strand breaks is much greater when the irradiation is delivered to human fibroblasts in anoxic rather than oxic conditions (M.C.P. and B.P.S., unpublished). We therefore maintained cultures in an N_2 atmosphere during irradiation, thereby greatly amplifying the signal-to-noise ratio in both repair assays.

Based on current insight into DNA repair mechanisms[13,18], a simple interpretation of our complementary data is that AT cells lack the full complement of functional γ endonuclease and thus fail to initiate excision repair of γ-induced base defects normally. We cannot, however, exclude other less direct explanations including the possibility that the AT strains carry a mutation affecting a later step in the repair process, thereby impeding the normal action of an otherwise unaltered endonuclease activity.

The results of our experiments have important practical and theoretical ramifications, especially in the fields of radiology and molecular radiobiology. Five, in particular, merit brief comment: (1) An inherited deficiency in a specific DNA repair mechanism is indicated as the molecular basis of ataxia telangiectasia. Aside from offering a reasonable and direct explanation for the reported clinical radiosensitivity of AT patients[6–8], our data provide new insight into the cause of this congenital disorder and, although admittedly problematic at present, may ultimately indicate the direction to follow in remedial treatment. (2) AT (ref. 2), like xeroderma pigmentosum[18] (XP), is a congenital disorder in which afflicted individuals are predisposed to an elevated incidence of malignancy, providing additional evidence that defective DNA repair has an aetiological role in at least some forms of neoplastic transformation[18,24]. (3) AT patients evidently defective in excision repair of radiation-damaged DNA bases are at an adaptive disadvantage; thus, it logically follows that a proficient mechanism to repair these radioproducts is of vital importance to the survival and reproductive capacity of humans. (4) AT strains are proficient in excision repair of ultraviolet photoproducts but defective in excision repair of γ radioproducts, whereas the reverse is found in XP strains[18]. These observations are consistent with a working model for this process in which the initial incision step is carried out by different enzymes, γ endonuclease or ultraviolet endonuclease, depending on the type of damage, whereas the remaining steps are executed by common enzymes. A similar model has been proposed for bacterial systems[22,23]. (5) Considerable success has been achieved in elucidating the excision-repair process active on ultraviolet photoproducts in DNA of human systems[18] after the discovery that XP fibroblasts are defective in ultraviolet-stimulated repair replication[24]. Since AT seems to be a γ-ray analogue of XP, it might be expected that comparable advances in our understanding of excision repair of γ radioproducts will be forthcoming.

We thank Drs A. R. Lehmann and A. M. Marko for discussions and encouragement; Drs A. M. R. Taylor and D. I. Hoar for preprints of articles[10,11]; and Drs D. R. Champ and N. E. Gentner for critical commentary on the manuscript. During the preliminary stages of this research P.H.M.L. was at Chalk River as an A.E.C.L. visiting scientist, and A.K.A. (Waterloo University, Waterloo, Ontario) and L.F. (McGill University, Montreal, Quebec) were undergraduate summer students.

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Sister chromatid exchanges in ageing and repair-deficient human fibroblasts

THE nature of the involvement of DNA repair in the formation of sister chromatid exchanges (SCEs) and their connection with chromosome aberrations is unknown. Some mutagens that damage DNA, including ultraviolet light and mitomycin C, considerably increase the incidence of SCEs[1–6], whereas other agents, such as X rays[6–8], nitrosoguanidine[2] and 8-ethoxycaffeine[5] cause only a slight increase, although they are potent inducers of lesions at the DNA and chromosome levels. Hamster cells infected with adenovirus 12 show no increased SCEs in spite of extensive fragmentation of the entire chromosome complement (our unpublished results). These findings suggest that the mechanism of formation of SCEs is not unitary. Whereas the mechanism of induction of SCEs could be studied by analysing their frequency after application of agents with known action on DNA, spontaneously occurring SCEs can be studied in cells with defects in the DNA repair system. This report deals chiefly with the frequency of spontaneous SCEs in several strains of xeroderma pigmentosum (XP) fibroblasts which show various levels of repair capacities, in Fanconi's anaemia (FA) cells and in control human cells at different stages of ageing.

We used three XP cell strains with different DNA repair capacities[9–11], three FA cell strains from unrelated patients and three fibroblast strains from unaffected subjects. All cultures were grown in Eagle's minimal essential medium supplemented with 15% foetal calf serum, and they were used for experiments at their earlier passages. To study SCE, cultures were treated for 38–42 h with 5-bromo-2′-deoxyuridine (BUdR) at various concentrations and fixed for chromosome preparation after 1 h of treatment with either colchicine or Colcemid. Chromosome preparations were made by the air-drying method and stained with acridine orange[2]. SCEs were scored under fluorescence microscopy.

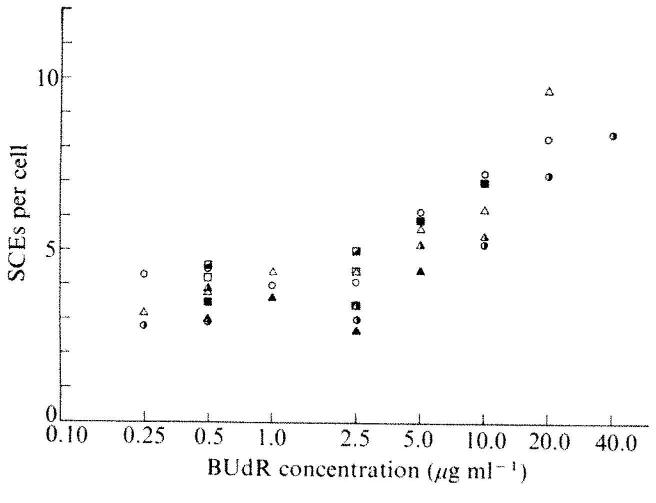

Fig. 1 Effects of BUdR on the frequency of SCEs in various human fibroblast strains. PEG (○) and BRU (half-shaded circle) were control strains established from skin punch biopsies of normal subjects. XP-E (△) and XP-C (half-shaded triangle) were obtained from skin biopsies of two unrelated XP patients and showed 21% and 32%, respectively, of normal unscheduled DNA synthesis after ultraviolet irradiation[6,8]. XP 1162 (▲) was obtained from the American Type Culture Collection (ATCC). This strain has been reported to have a normal repair capacity as measured by unscheduled uptake of ^{3}H-thymidine after ultraviolet irradiation. FA 122 (□) was an ATCC strain. FA 40400 (half-shaded square) and FA 15100 (■) were gifts from Dr Siminovitch University of Toronto. Each point was determined based on 10–25 cells which had been collected at the 2nd metaphase after labelling and stained with acridine orange (0.125 mg ml^{-1}).

Table 1 The frequency of sister chromatid exchanges in chromosomes of various human fibroblasts labelled with BUdR at concentrations lower than 2.5 μg ml^{-1}

Cell strains (sex)	No. of cells observed	No. of SCEs scored	SCEs per cell (± s.e.m.)	Cells with chromosome aberrations† (%)
Normal				
PEG (F)	90	382	4.24±0.22	—
BRU (M)	60	193	3.22±0.23	1.8
HE-1* (F)	350	1,325	3.78±0.10	1.2
Xeroderma pigmentosum				
XP-E (F)	57	249	4.37±0.28	2.9
XP-C (M)	80	289	3.61±0.21	1.5
XP 1162 (M)	30	91	3.03±0.32	0
Fanconi's anaemia				
FA 122 (M)	40	173	4.33±0.33	13.6
FA 40400 (F)	27	130	4.81±0.42	11.0
FA 15100 (M)	50	169	3.38±0.26	15.0

*Embryonic fibroblast strain at passage 12 (a gift from Dr Nakagome of National Institute of Genetics, Mishima). Exact ages of other cultures are uncertain but all were used for experiments within 2–3 passages after thawing of frozen stocks prepared earlier at passages after initiation or purchase of the cultures.

†Determined on 70–200 cells each. There is no statistically significant difference in the aberration frequencies between the normal and XP cells as well as among strains in each group, while the frequencies obtained for all FA cells differ significantly from those in cell strains in the other two groups ($P < 0.01$). Most aberrations detected in FA cells as well as in the other strains were chromatid gaps and breaks.

Figure 1 shows that SCE frequency varied only slightly in each cell strain after treatment with BUdR in concentrations ranging from 0.25 to 2.5 μg ml^{-1}, confirming previous results[2,4]. We refer to this plateau level as spontaneous SCE although we cannot exclude the possibility that even these exchanges resulted from the application of BUdR[12]. To compare the frequencies of SCEs in various cell strains, samples were treated with BUdR at concentrations less than 2.5 μg ml^{-1}. There was no major difference in SCE frequency among fibroblast strains (Table 1). Irrespective of the DNA repair capacity of the cell, SCE frequencies varied between 3 and 4.5 per diploid chromosome complement. It is interesting that FA cells, in spite of high incidence of spontaneous chromosome aberration (Table 1) showed no increased level of sister chromatid exchanges. This observation suggests that, at least in the FA cells examined, the formation of spontaneous chromosome aberrations is dissociated from processes leading to SCE. XP cells seem to have a reduction in the endonuclease activity which is necessary to initiate excision repair[13]. But a class of XP, the XP varient, is characterised by its normal excision repair and suspected to be defective in post-replication repair[14]. FA cells are believed to be defective in some DNA repair mechanisms[15,16], probably lacking one or more enzymes[17]. Our present results are consistent with previous reports on the yield of SCE in XP[18] and FA cells[19]. The genesis of spontaneous SCEs may be independent of these particular repair mechanisms.

The spontaneous exchange frequency seems to be affected by the age of cell culture. The normal human fibroblast strain HE-1, at passage 12, was used as a starting culture and grown for about two months; its SCE frequency was examined periodically. To minimise division delay caused by frequent subculturing, we used a 1:6 split scheme at 3 or 4-d intervals during the first month of culture and at

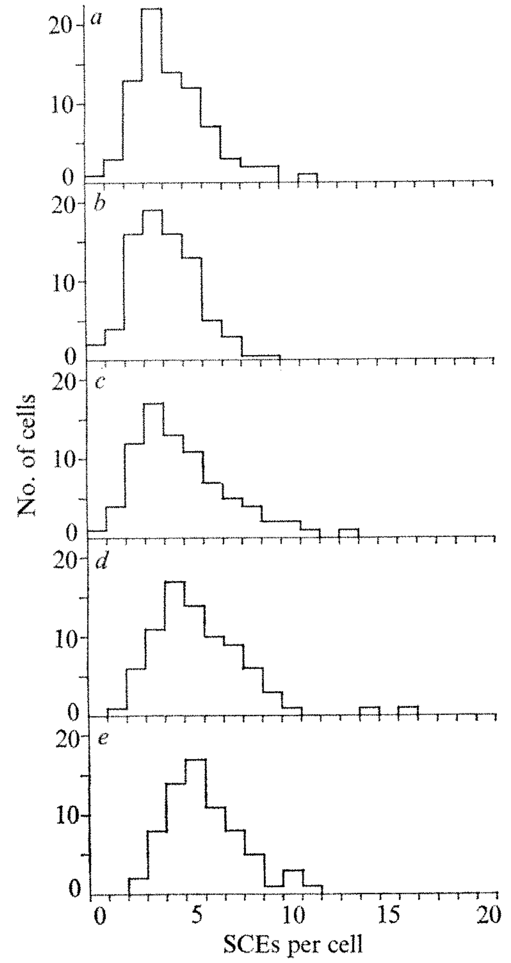

Fig. 2 The shift in SCE frequency during long term culture of HE-1. According to the subculturing scheme used, the culture is considered to undergo about two and a half population doublings per passage. Passages 13(*a*), 19(*b*), 24(*c*), 27(*d*) and 30(*e*) may therefore correspond roughly to 33, 48, 60, 68 and 75 population doublings, respectively. Cultures were treated for 40 h with BUdR (0.25 μg ml^{-1}) before collection. Mean ± s.e.: *a*, 3.99±0.22; *b*, 3.63±0.21; *c*, 4.46±0.24; *d*, 5.28±0.26; *e*, 5.41±0.26.

longer intervals thereafter. According to this scheme each passage would correspond roughly to 2.5 population doublings. In these conditions, cells ceased growth after passage 32, about 80 d after the start of the experiment. A statistically significant increase in SCE frequency was observed during long term culture but only at the end of the life span of the culture. As Fig. 2 shows the modal value of the exchange frequency per diploid chromosome complement was 3 at the onset of the experiment (at the 33rd population doubling level). This value remained unchanged up to passage 24 (60 doublings), then moved to 4 at passage 27 (68 doublings) and reached 5 at passage 30 (at the 75th population doubling level). At this last period the cell cultures contained many slow growing cells which showed several symptoms of senescence, including considerable variation in size and morphology and an increased level of chromosome aberration. The incidences of cells having chromosome breaks and exchanges were 1.2%, 2.1%, 5.3% and 9.6% at passages 13, 24, 27 and 30, respectively. This increased production of aberrations during the latest part of the life span seems to be compatible with the finding of Saksela and Moorhead's findings[20] with WI-38 cells, and parallels the increase in the SCE frequency.

The results obtained with XP and FA cells obtained in this study support the conclusion[18] that the induction of spontaneous SCE cannot be explained by a simple application of concepts from known DNA repair mechanisms. On the other hand, the observed link between SCE and chromosome aberrations in the ageing cell culture and in Bloom's syndrome[19] and their dissociation in FA cells indicate that the pathways through which both events arise are at least partially different.

Part of this study was carried out while H. K. was at the University of British Columbia at the Laboratory of H.F.S. We thank Miss C. Yoshizawa for technical assistance. This study was supported in part by the National Research Council of Canada and the National Cancer Institute of Canada and by a grant from the Ministry of Education, Science, and Culture of Japan.

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Received September 15, 1975; accepted February 9, 1976.

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Differential chromatid staining by *in vivo* treatment as a mutagenicity test system

DIFFERENTIAL staining of sister chromatids can be demonstrated with Hoechst 33258 fluorochrome[1] and with Giemsa after special pretreatment[2], even if 5-bromodeoxyuridine (BUdR) is present during the first S phase only[3]. These techniques permit the use of the phenomenon of the sister chromatid exchange (SCE). The average spontaneous rate of 10–14 SCEs per metaphase seems to be fairly constant in all cultures from several species[4,5]. A variety of mutagenic factors (that is, ultraviolet light and alkylating agents) produce very high frequencies of SCE even at concentrations far below the level necessary for the induction of chromosomal breakage, whereas others, like X rays or certain chemicals, hardly influence the rate of SCE[6]. Because of the high turnover of BrdU in the liver and the physiologically available thymidine, differential chromatid staining has so far only been produced in *in vitro* systems (cell cultures) and in the chicken embryo.

We have found that the additional application of 5-fluorodeoxyuridine (FUdR) which inhibits thymidine synthesis leads to differential chromatid staining in metaphases prepared directly from bone marrow. This method proved to be a sensitive test system for mutagenicity as demonstrated by the increasing frequencies of SCE after application of two alkylating agents.

Mature mice were injected with 0.4 mg FUdR per g body weight intraperitoneally; beginning 3 h later they received

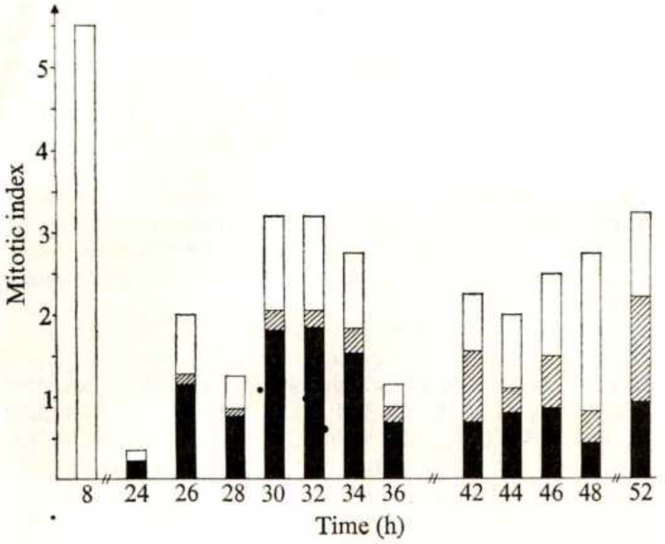


Fig. 1 Solid column, second mitoses (complete differential chromatid staining); hatched column, third mitoses.

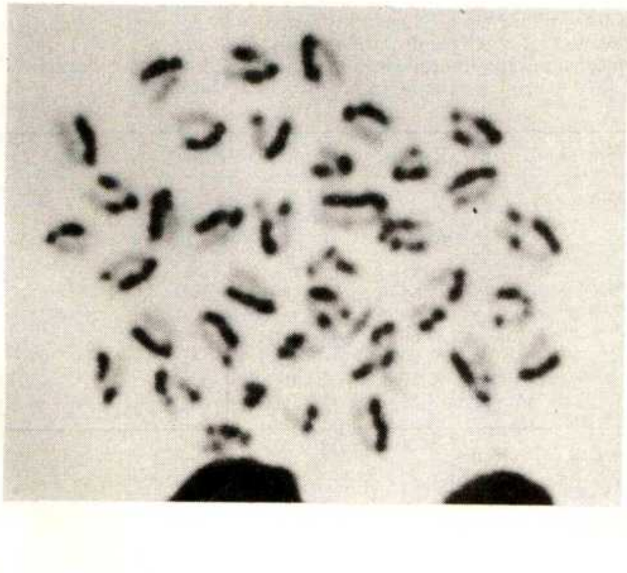
six times 0.04 mg BUdR per g body weight during 6 h. Colcemid (16 μg) was administered 3 h before killing. Metaphases from bone marrow were prepared using standard methods without culturing, and stained according to Epplen *et al.*[8]. Mitotic indices and frequencies of differential chromatid staining in the second and the following mitoses are given in Fig. 1. The highest incidence of second mitoses appears about 30 h after the first BUdR injection. About 2 h after BUdR treatment a replication pattern can be found.

Table 1 SCE per metaphase after administration of various concentrations of triaziquon and cyclophosphamide

μg per g body weight	SCE per metaphase
Triaziquon	
0	4
0.0025	6
0.0125	10
0.125	30
Cyclophosphamide	
0	4
12.5	15
25	24

Each value represents at least 20 metaphases of two different specimens.

Fig. 2 Bone marrow metaphase with differential chromatid staining showing a high incidence of SCE after *in vivo* treatment of the mouse with cyclophosphamide.



Thus a method has been developed with the capacity to induce differential chromatid staining by *in vivo* treatment. Compared with *in vitro* studies the 'spontaneous' rate of SCE turned out to be extremely low (4 per metaphase). Possible explanations for this difference may include, for example, the complete absence of light from the cells in the bone marrow or a lower mean concentration of BUdR during treatment. To demonstrate the efficiency of the method for mutagenicity testing we have studied the induction of SCE by two alkylating agents *in vivo*. Both substances produce a dose-dependent increase in SCEs (Table 1) which can be detected at concentrations below those inducing chromosomal breakage (Fig. 2). Using higher concentrations, the mitotic index and the proportion of differentially stained metaphases is greatly reduced by the toxic activity of the drugs. As to be expected, the effect of cyclophosphamide is evident in our *in vivo* system, whereas it was inert in *in vitro* studies[6]. In so far as the induction of SCE can be regarded as an indication for mutagenicity[9], our method enables the application of this test system to *in vivo* studies.

Since *in vitro* systems of mutagenicity testing are subject to methodical errors, the transfer of these systems into physiological conditions, that is, *in vivo*, is preferable. If the restrictions with respect to the significance of SCE are borne in mind[9], our method may turn out to be a useful tool in mutagenicity testing.

T.B. is in receipt of a trainee fellowship from the Deutsche Forschungsgemeinschaft.

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Received January 12; accepted February 10, 1976.

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Analysis of sister chromatid exchange formation *in vivo* in mouse spermatogonia as a new test system for environmental mutagens

SPERMATOGONIA, as stem cells of male gametes[1,2], constitute a highly relevant system for studying the genetic hazards of potential mutagens. Gross chromosome aberrations in spermatogonia have been used as a gauge of the genetic damage induced in animals exposed to agents such as mitomycin C (refs 3–5). Sister chromatid exchanges (SCEs) formed in response to DNA damage constitute even more sensitive indices of the impact of alkylating agents and other clastogens on chromosomes[6–8]. Analysis of SCE induction in cultured cells has been greatly facilitated by techniques in which substitution of DNA with the base analogue 5-bromodeoxyuridine (BUdR) is detected either with fluorescent dyes[9,10] or by modified Giemsa procedures[11–13]. This approach has so far been limited to *in vitro* trials, with the exception of one system[14] in which SCEs were visualised in chromosomes of chick embryos after exposure to BUdR *in ovo*. We report here a BUdR technique which enables the detection of SCEs formed in spermatogonial cells of intact mice. As a prototype of a

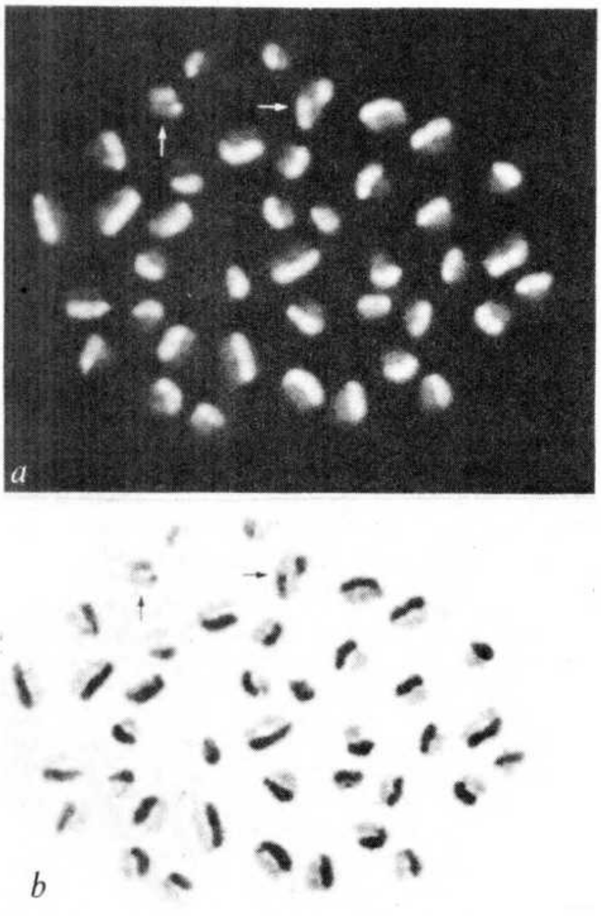

Fig. 1 Sister chromatid differentiation and SCEs in mouse spermatogonia. After *in vivo* administration of BUdR and slide preparation as described in the text, this cell was stained with 33258 Hoechst. *a*, Result of fluorescence microscopy; *b*, appearance of the same cell following subsequent Giemsa staining. SCEs indicated by arrows.

general *in vivo* mutagenesis test, this procedure has been utilised to demonstrate a several-fold increase in the sister chromatid exchange frequency in mouse spermatogonia after injection of the animals with small amounts of mitomycin C.

Substitution of spermatogonial chromatids in 3–5-month-old male CBA/J mice was effected by administering 14 hourly intraperitoneal (0.2 ml) injections of BUdR (10^{-2} M) together with deoxycytidine (5×10^{-3} M), the latter to reduce BUdR toxicity[15]. The series of injections served to replenish exogenous nucleotide which is thought to undergo rapid depletion *in vivo*[16]. The total duration of exposure to BUdR (approximately 14 h) is presumed sufficient for incorporation of this base analogue into all spermatogonial subpopulations, including the predominating type B spermatogonia which exhibit the longest DNA synthesis periods[17]. Mitomycin C, when administered, was injected as a single intraperitoneal dose (0.025–0.5 mg kg^{-1}) 26 h after the initial injection of BUdR. After an additional 28-h period, Colcemid was injected (0.6 mg kg^{-1}, intraperitoneally) and the animals were killed by ether narcosis 3–5 h later. Spermatogonial chromosomes were prepared from isolated seminiferous tubules[18], stained with 33258 Hoechst[9,19], and sister chromatid differentiation examined by fluorescence microscopy which, in many cases, was followed by Giemsa staining [11,12].

Most of the metaphase cells examined revealed the differential chromatid fluorescence characteristic of 33258 Hoechst stained cells which had replicated twice after administration of BUdR[20] (Fig. 1). Similar patterns could be observed with Giemsa staining after the intense illumination used for fluorescence microscopy[12]. In view of the schedule of BUdR administration and the generation times of the spermatogonial cells[17], the present sister chromatid differentiation is believed to reflect one cycle of BUdR

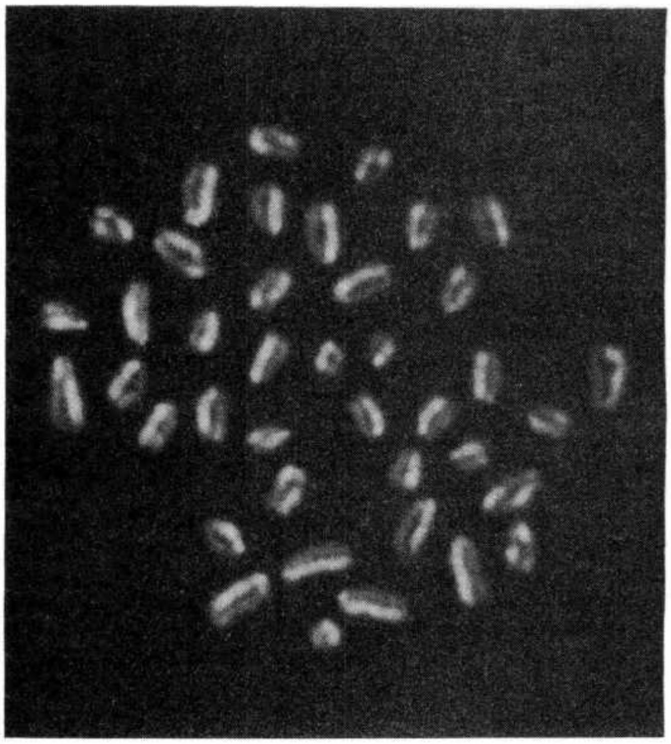

Fig. 2 Extensive induction of SCEs in a mouse spermatogonial cell by mitomycin C. This cell was treated as described in Fig. 1, except that a single dose of mitomycin C (0.5 mg kg^{-1}) was injected intraperitoneally into the animal 32 h before collection. Eighteen SCEs are evident in this cell.

Table 1 SCEs and chromosome aberrations induced by mitomycin C in BUdR-substituted spermatogonia

Mitomycin C dosage (mg kg*)	Mice	Metaphases†	Genomes‡	SCEs	SCEs per genome	Aberrations§ per genome
0 (control)	4	202	261	473	1.81	0.01
H_2O (control)	2	98	121	200	1.65	0.02
0.025	3	77	95	199	2.09	0.00
0.05	4	125	156	562	3.60	0.04
0.10	4	114	140	506	3.62	0.03
0.30	4	132	179	863	4.82	0.03
0.50	3	78	99	716	7.23	0.02

*Injections (approximately 0.3 ml) were given approximately 31–33 h before cell collection.
†Diploid- and polyploid-appearing cells.
‡A diploid genome contained 40 chromosomes.
§Aberrations included translocations, chromatid breaks and quadriradial figures.

incorporation followed by a second cycle in the relative absence of analogue. Metaphase cells collected at earlier times (23 h after initiation of BUdR administration) and stained with 33258 Hoechst demonstrated the lateral fluorescence asymmetry expected for satellite DNA containing centromeric regions which have undergone one round of BUdR incorporation[21]. An occasional second division cell exhibited a longitudinal fluorescence differentiation, reflecting BUdR incorporation for only part of a DNA synthesis period, thereby suggesting usefulness of this method for studying replication kinetics.

An average of somewhat less than 2 SCEs per cell were observed for control animals given BUdR but not mitomycin C (Table 1). Frequencies are normalised for the numerous multiple genome figures reflecting the interconnected cell populations characteristic of male germinal tissue[22]. The SCE rate increased to two, three and four times that of control at mitomycin C doses of 0.05, 0.3, and 0.5 mg kg^{-1}, respectively. The last dose, which markedly increased the exchange frequency (Fig. 2), induced fewer than 0.1 morphological aberrations (breaks, rearrangements) per second division metaphase. Consistent with other reports[3,23], higher doses of mitomycin C (1.0–5.0 mg kg^{-1}) markedly reduced the number of metaphase cells available for analysis, presumably by retarding the proliferation of severely damaged spermatogonia. Preliminary observations using the techniques described above indicate that intraperitoneal injection of mitomycin C is also capable of inducing SCEs in mouse bone marrow cells.

The use of intact animals in the present study provides a highly relevant test system which takes into account host metabolism and possible activation of the drug[24,25], as well as passage of the drug across the blood–testis barrier[26]. Technical improvements in this procedure, in particular those simplifying the administration of BUdR to animals, should facilitate application of this approach. The precise relationship between SCE induction and mutagenesis remains to be determined, although the two events exhibit a high correlation[8]. Mitomycin C-induced chromosome rearrangements in spermatogonia have already been implicated in mutagenesis[4,27] and errors potentially associated with SCE formation may similarly contribute to the mutation frequency[28]. Thus, SCE analysis in mammalian spermatogonia should constitute an important new test of environmental mutagenesis.

An additional potential of a spermatogonia cell system for BUdR-dependent differentiation between sister chromatids is its natural extension for experimental studies of meiosis. If collection of the seminiferous tubules is delayed for approximately 2 weeks after BUdR administration, BUdR and therefore sister chromatid differentiation might be present in condensed meiotic chromosomes[29]. Optical detection of chromatid exchange between homologues should then enable high resolution analysis of meiotic recombination, which could also be compared with SCE frequencies in different cell types of the same animals

Supported by the National Institutes of Health and the American Cancer Society. 33258 Hoechst was a gift from Dr H. Loewe.

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Received February 2, accepted February 18, 1976

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Fate of thiazolidine ring during fragmentation of penicillin by exocellular DD-carboxypeptidase-transpeptidase of *Streptomyces* R61

LIKE various β-lactamases, acylases and esterases[1], the exocellular DD-carboxypeptidase-transpeptidase of *Streptomyces* R61 degrades benzylpenicillin and other β-lactam antibiotics[2–4]. The R61 enzyme, however, markedly differs from the other penicillin-degrading enzymes in causing fragmentation of the penicillin nucleus. By using 8-^{14}C-benzylpenicillin (benzyl labelled) as substrate, one of the fragments produced was shown to be ^{14}C-phenylacetylglycine[5]. The reaction with the R61 enzyme is also peculiar in that it is a slow process. This is because of the long half life of the stoichiometric complex transitorily formed between the antibiotic and the enzyme. Thus, for example, the value of the half life for the complex formed with benzylpenicillin is 80 min in 10 mM phosphate buffer (*p*H 7.0) and at 37 °C. As breakdown of the complex proceeds, however, phenylacetylglycine (when benzylpenicillin is used as substrate) is released and the enzyme concomitantly recovers its ability to bind penicillin. We have now characterised the fragment (hereby designated as the Y product) arising from the thiazolidine ring of penicillin as a result of the fragmentation of the antibiotic molecule by the R61 enzyme.

The large amount (about 20 μmol) of ^{14}C-phenylacetylglycine previously produced from 8-^{14}C-benzylpenicillin through a series of enzyme inactivation and reactivation (total time for the operation, 100 h) could be purified by filtration of the reaction mixture on a column of Sephadex G-25 on the basis that phenylacetylglycine was eluted at a volume slightly larger than the salt volume[5]. Examination of the fractions containing ^{14}C-phenylacetylglycine by NMR spectrometry failed to reveal any lines that could be attributed to methyl groups. In marked contrast, the NMR spectrum in D_2O of the non-radioactive material obtained from the salt-containing fractions exhibited lines characteristic of methyl groups. The spectrum indicated that the thiazolidine ring had been opened and suggested that the split product was either *N*-formyl-D-penicillamine or di-(*N*-formyl-D-penicillamine)disulphide (Fig. 1). Direct chromatography of a sample of this fraction on Aminex A5 ion-exchange resin yielded only very minor ninhydrin-positive peaks. Mild acid hydrolysis (1 h in 1 N HCl at 60 °C, that is, in conditions which are known to release the formyl group from di-(*N*-formyl-D-penicillamine)disulphide[6]) produced one important ninhydrin-positive compound which had the same behaviour on the Aminex column as di-(D-penicillamine)-disulphide. Finally, mild acid hydrolysis followed by performic oxidation yielded a highly acidic ninhydrin-positive product which, on the same Aminex column, was indistinguishable from the product obtained by performic oxidation of D-penicillamine.

In order to characterise the Y product further and to study the kinetics of its release, various radioactive penicillins with the radioactive label in the thiazolidine portion of the molecule were prepared. ^{3}H-Benzylpenicillin (labelled in the β-methyl

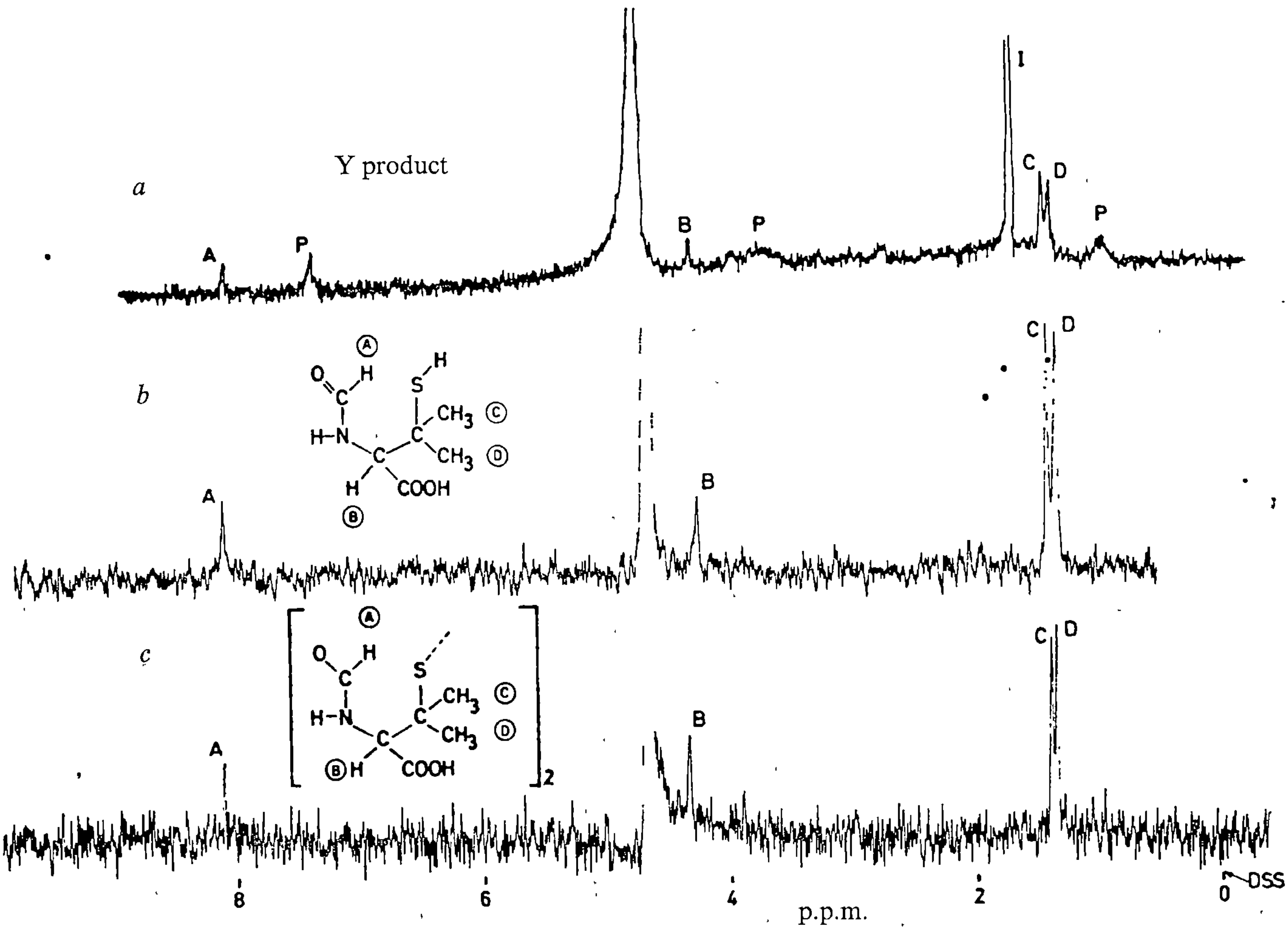

Fig. 1 NMR spectra in D_2O of Y product (*a*), *N*-formyl-D-penicillamine (*b*) and di-(*N*-formyl-D-penicillamine) disulphide (*c*). The spectra were obtained with a Varian XL100-15 spectrometer operated in the ^{2}D internal lock mode. The I line exhibited by the Y preparation (*p*H 7.0) was attributed to an impurity and the P lines were attributed to the presence of a small amount of penicilloic acid. The A, B, C and D lines, occurring in the ratios of 1: 1: 3: 3, were attributed to the Y product The 6 Hz distance between the methyl lines (C and D) excluded the presence of an intact thiazolidine ring (in which case the distance value should have been 38 Hz) The spectra of the Y product, *N*-formyl-D-penicillamine and di-(*N*-formyl-D-penicillamine)disulphide were virtually superimposable with respect to lines A (8.09, 8.11 and 8.09 p.p.m., respectively), B (4.31, 4.28 and 4.34 p.p.m., respectively), C (1.40, 1.38 and 1.36 p.p.m., respectively) and D (1.46, 1.45 and 1.41 p.p.m., respectively). The p.p.m values are relative to 2,2-dimethyl-2-silapentane-5-sulphonate (DSS). Further confirmation was obtained by extracting the Y product (after acidification) with ethylacetate. The extract was evaporated to dryness and the residue dissolved in $CDCl_3$ in the presence of dimethylsulphoxide Proton B of the CH group located in the vicinity of the NH group gives a characteristic doublet (9 Hz splitting) The SH proton, however, could not be seen because of the presence of a large impurity peak in its resonance region and it was therefore impossible to decide whether the Y product was *N*-formyl-D-penicillamine or di-(*N*-formyl-D-penicillamine)disulphide. The *N*-formyl-D-penicillamine used as standard (spectrum *b*) was prepared by dissolving D-penicillamine HCl (550 mg) in 7 ml formic acid (95%) containing 200 mg of sodium formiate After addition of 2 ml of acetic anhydride, the reaction proceeded for 1 h at room temperature. Melting point 146–148 °C; (α^{20}_{D}+63 (C= 1, pyridine)). The optical rotation is in agreement with the value published previously[6]. The di-(*N*-formyl-D-penicillamine)disulphide (spectrum *c*) was prepared from *N*-formyl-D-penicillamine by oxidation in air in the presence of FeCl[6].

group, 1.7 mCi mmol^{-1}), 5-^{14}C-benzylpenicillin (0.07 mCi mmol^{-1}) and 5-^{14}C phenoxymethylpenicillin (that is, penicillin V; 0.08 mCi mmol^{-1}). As determined by the bioassay and iodometric assay, the purity of the compounds was higher than 98%. Thin-layer chromatography showed, besides the penicillin spot, a small amount of penicilloic acid (±2%) but no other radioactive impurities.

In a first series of experiments, maximal amounts of ^{3}H-Y and ^{14}C-Y products were prepared from ^{3}H-benzylpenicillin and 5-^{14}C-benzylpenicillin, respectively. In these assays, the R61 enzyme was maintained saturated by successive additions of radioactive penicillins to the reaction mixtures. The total period of time for the whole operations was 24 h and a total amount of 0.5 μmol of each radioactive benzylpenicillin was converted into degradation products. The accumulated ^{3}H-Y and ^{14}C-Y products were partially purified by filtration on Sephadex G-25 The following observations were made. (1) Both radioactive Y products had the same *Rf* values by thin-layer chromatography and with the two solvents used, these *Rf* values were identical to those of di-(*N*-formyl-D-penicillamine)-disulphide (Table 1). (2) At least 95% of the radioactive labels originally engaged in the reactions, cochromatographed on the Aminex A5 column exactly with exogenously added, non-radioactive di-(*N*-formyl-D-penicillamine)disulphide (as revealed by ninhydrin after acid hydrolysis of a sample of the fractions obtained; Fig. 2*a*). (3) Treatment of the ^{3}H-Y product with 1 N HCl for 60 min at 60 °C produced (95% yield) a radioactive compound which on the Aminex column cochromatographed exactly with exogenously added, non-radioactive di(D-penicillamine)disulphide (as revealed directly with ninhydrin; Fig. 2*b*). (4) HCl Treatment of the ^{14}C-Y product in the same conditions as above yielded a non-radioactive compound which also behaved on the Aminex column as di(D-penicillamine)disulphide (and was directly revealed with ninhydrin). Since the ^{14}C-Y product was formed by action of the R61 enzyme on 5-^{14}C-benzylpenicillin and since treatment of the ^{14}C-Y product under conditions known to release the formyl group from di-(*N*-formyl-D-penicillamine)disulphide

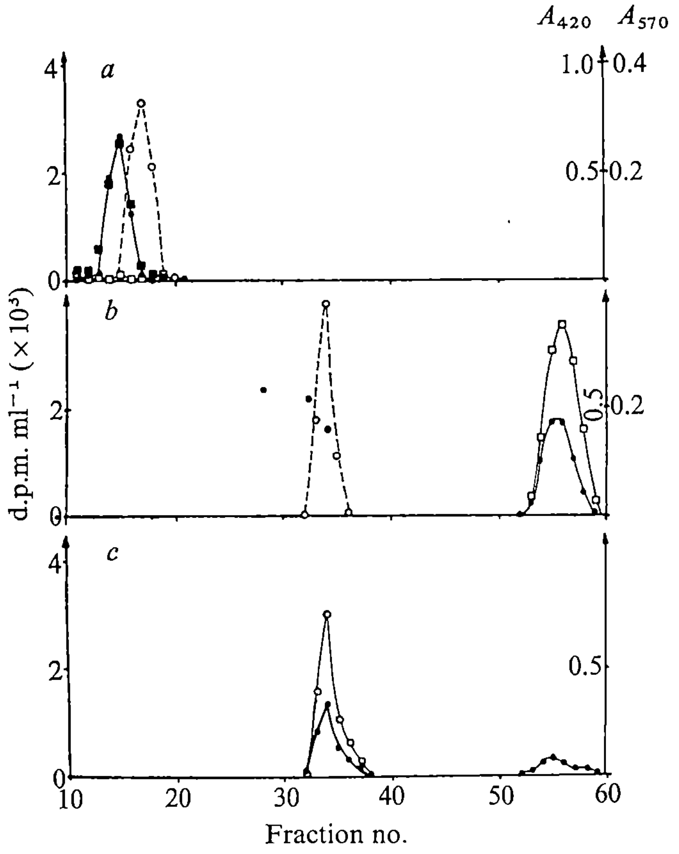

Fig. 2 Identification by chromatography on Aminex A5 of the Y products released from benzylpenicillin after interaction with the R61 enzyme under prolonged (*a* and *b*) and short (*c*) incubation times. The column (9×540 mm) was equilibrated against 0.2 M sodium citrate–HCl buffer (*p*H 3.17). Elution was performed with the same buffer at a flow rate of 80 ml h^{-1} (volume of the fractions: 3.2 ml). *a*, A mixture containing 10 nCi of ^{3}H-Y product and 200 μg of each *N*-formyl-D-penicillamine and di-(*N*-formyl-D-penicillamine)disulphide was deposited. Radioactivity (●) was measured on 1-ml samples. The *p*H of 0.4-ml samples was adjusted to 7.5–8.0 by addition of 40 μl of 2 M Tris base and the free sulphydryl groups (○) were estimated by adding 20 μl of 50 mM Tris-HCl buffer (*p*H 8.0) containing 40 μg of 5,5′-dithio-*bis*(2-nitrobenzoic acid) (A_{420}). The free amino groups were estimated by reaction with ninhydrin (A_{570}) before (□) and after (■) a 60 min incubation at 60 °C in 1 N HCl. *b*, A mixture containing 10 nCi of ^{3}H-Y product and 400 μg of di-(*N*-formyl-D-penicillamine)disulphide was incubated at 60 °C for 60 min in 1 N HCl. The *p*H was then adjusted to 2.2, 200 μg of D-penicillamine were added and the mixture was deposited on the column. Radioactivity (●) was measured on 1-ml samples. Free sulphydryl groups (○) and free amino groups (□) were estimated on 0.4-ml and 0.5-ml samples, respectively, as in *a*. *c*, Chromatography of the HCl-hydrolysed tritiated compound prepared by a 2-h incubation of ^{3}H-benzylpenicillin with the R61 enzyme (for conditions, see text) The radioactivity (●) was measured on 1-ml samples. Free sulphldryl groups (○) were estimated as above. The A_{570} value after colour development of D-penicillamine with ninhydrin is about 7% of that obtained with equivalent amount of D-penicillamine disulphide.

actually resulted in the release of the radioactive label, it seems clear that the formyl group of di-(*N*-formyl-D-penicillamine)-disulphide arises from C5 of the penicillin nucleus. (5) Thin-layer chromatography in solvent B (for composition, see Table 1) of the hydrolysed ^{3}H-Y and ^{14}C-Y products revealed the presence of a radioactive ninhydrin-positive spot and of a non-radioactive ninhydrin-positive spot, respectively, exhibiting the same *Rf* value (0.27) as di-(D-penicillamine)disulphide. (6) Finally, performic oxidation of the HCl-treated ^{3}H-Y product yielded a very acidic ^{3}H compound (90% yield) which, on the Aminex column, cochromatographed exactly with exogenously added penicillamine previously oxidised with performic acid.

In a second series of experiments, a half life of 90 min for the ^{3}H-benzylpenicillin–R61 complex (in 10 mM phosphate buffer (*p*H 7.0) and at 37 °C) was determined on the basis of the release of the radioactive label. In previous studies[3], an average half life of 80 min had been determined for the complex formed with the R61 enzyme. Within the limits of experimental errors therefore, recovery of enzyme activity and release of the two fragments arising from the benzylpenicillin molecule occur with the same rate constant (1.4×10^{-4} s^{-1}). This result together with the facts that after complete enzyme recovery, more than 90% of the original radioactive labels were recovered as ^{14}C-phenylacetylglycine and either ^{3}H- or ^{14}C-di-(*N*-formyl-D-penicillamine)disulphide (depending on the radioactive benzylpenicillins used), show clearly that phenylacetylglycine and di-(*N*-formyl-D-penicillamine) are produced in stoichiometric amounts.

Since the Y product characterised as di-(*N*-formyl-D-penicillamine)disulphide was isolated after several cycles of enzyme inactivation and reactivation (that is, after prolonged incubation), we wondered whether it was actually the one initially released through breakdown of the penicillin molecule. In a third series of experiments, two samples, each containing 5 nmol of ^{3}H-benzylpenicillin and 6 nmol of R61 enzyme, were incubated for 2 h at 37 °C under nitrogen. Reaction of one of the samples with 5,5′-dithio-*bis*(2-nitrobenzoic acid) indicated free SH groups. Non-radioactive *N*-formyl-D-penicillamine (125 μg) was added to the second sample and the solution was treated with 1.2 N HCl (final concentration) for 16 h at 60 °C, that is in conditions where release of the formyl group from *N*-formyl-D-penicillamine was known to occur[6]. Chromatography on Aminex A5 revealed that 80% of the radioactive product present in the hydrolysate was penicillamine (Fig. 2*c*). About 20% of penicillamine disulphide was detected. Thus *N*-formyl-D-penicillamine was probably the primary product released and di-(*N*-formyl-D-penicillamine)disulphide was a product subsequently formed by oxidation. Surprisingly, however, by incubating 50 nmol of *N*-formyl-D-penicillamine for 16 h at 37 °C, in 100 μl of 10 mM phosphate buffer (*p*H 7.0), either as such, or in the presence of 0.5 nmol of heat-inactivated R61 enzyme (5 min at 100 °C), or in the presence of the same amount of active R61 enzyme, it was observed that 80–90% of the SH groups originally present remained titratable by dithio-*bis*-nitrobenzoic acid after incubation in the absence of enzyme or in the presence of inactivated enzyme but that 80% of these groups had disappeared after incubation in the presence of native enzyme. Thus, it is not impossible that the enzyme preparation somehow participated in the oxidation of the *N*-formyl-D-penicillamine initially released.

Table 1 Thin-layer chromatography on silica gel (G) of radioactive Y product and of non-radioactive *N*-formyl-D-penicillamine and di-(*N*-formyl-D-penicillamine)disulphide

Compound	*Rf* values in solvents A	 B
^{3}H- or ^{14}C-Y product	0	0.69
N-formyl-D-penicillamine	0.34	0.68
di-(*N*-formyl-D-penicillamine)disulphide	0	0.67

Solvent A, $CHCl_3/CH_3OH/CH_3COOH$: 88/10/2 (v/v/v).
Solvent B, 1-butanol/$H_2O/C_2H_5OH/CH_3COOH$: 10/4/3/3(v/v/v/v).
Both the peaks of radioactivity (detected by scanning the thin-layer plates in a radiochromatogram scanner) and the spots of di-(*N*-formyl-D-penicillamine)disulphide (as revealed by spraying the plates with an alcoholic solution of bromocresol green) were perfectly symmetrical.

Comparable results were obtained with 5-^{14}C-phenoxymethylpenicillin which was degraded into ^{14}C-*N*-formyl-D-penicillamine and non-radioactive phenoxyacetylglycine.

In summary, it seems that within the limits of experimental

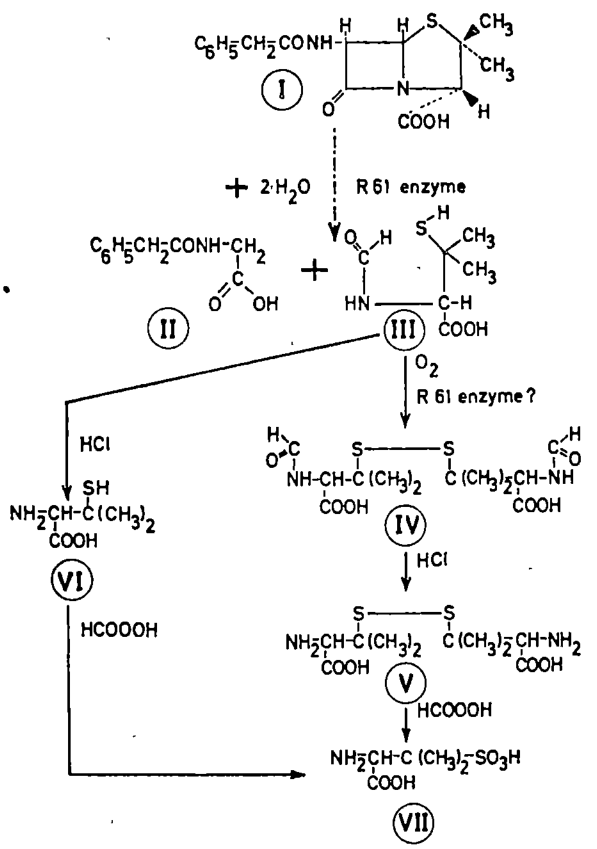

Fig. 3 Degradation of benzylpenicillin I, benzylpenicillin; II, phenylacetylglycine; III, N-formyl-D-penicillamine; IV, di-(N-formyl-D-penicillamine)disulphide; V, D-penicillamine disulphide; VI, D-penicillamine; VII, dimethyl cysteic acid. Degradation of III into VI requires acid treatment (1 N HCl) during several hours at 60 °C. Degradation of IV into V can be achieved by the same acid treatment during 1 h.

error, Nα-(acyl)-glycine and N-formyl-D-penicillamine are released in stoichiometric amounts from the complex formed between penicillin and the exocellular R61 DD-carboxypeptidase-transpeptidase, and that release of these fragments and enzyme reactivation are concomitant events. If penicillin is benzylpenicillin, phenylacetylglycine is released. If penicillin is phenoxymethylpenicillin, phenoxyacetylglycine is released. Globally, the reaction consists in the addition of two H_2O molecules and results in the hydrolysis of the amide bond and in the rupture of both C5–C6 and C5–S linkages (Fig. 3) The nature of the acyl substituent on the antibiotic molecule is important; although it does not modify the pathway of the reaction, it obviously influences the half life of the complex formed between penicillin and the enzyme The released N-formyl-D-penicillamine undergoes subsequent oxidation with formation of the disulphide derivative.

Studies[7,8] with isolated membranes have shown that enzyme–penicillin complexes found in membranes do not always give rise to the products we have found with purified enzyme. Further work is required in order to unravel the exact mechanism of the reactions.

This work was supported in part by the Fonds de la Recherche Fondamentale Collective, Brussels, Belgium. We also thank UCB, Brussels, Belgium, for financial support. J.M.F. and J.D. are Chargés de recherches of the Fonds National de la Recherche Scientifique, Brussels, Belgium.

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Received December 19, 1975, accepted January 16, 1976.

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Phage-coded protein prevents restriction of unmodified progeny T4 DNA

HOST-controlled restriction modification systems usually prevent the survival of unmodified genomes in bacterial cells[1–4]. Phage DNA can be modified by methylation (λ and f1)[5–7] or by glucosylation (T-even phages[3]). Modification of the λ genome is mediated by a host methylase[8] whereas modification of the T4 genome is by phage coded α- and β-glucosyl transferases[9]. The restriction systems of *E. coli* (*rgl*) acting on non-glucosylated (glu$^-$) T-even phages are called r_6 and $r_{2,4}$ (ref. 10): r_6 restricts all glu$^-$ T-even phages, whereas $r_{2,4}$ restricts only T2 and T4 glu$^-$ phages. Hattman and Fukasawa[11] showed that when glu$^+$ T4 DNA was injected into an *rgl*$^+$ uridine diphosphoglucose (UDPG) pyrophosphorylaseless mutant, the progeny glu$^-$ DNA replicated and was matured into virions which they called T*. To explain why the replicating glu$^-$ progeny DNA is not restricted in the *rgl*$^+$ host while the entering T* virion DNA is, Fukasawa[12] postulated that the *rgl* system is localised in the membrane. Incoming glu$^-$ parental DNA would be restricted during passage through the membrane. Incoming glu$^+$ parental DNA (which is not restricted) would be replicated as glu$^-$ DNA in the absence of UDPG. Since the replicating DNA is not subject to the restriction system localised in the membrane it should not be restricted. Not surprisingly, r_6 was shown to be localised in the membrane[13,14]. There is, however, good evidence that $r_{2,4}$ can restrict replicating DNA[15,16] and is, at least in that sense, cytoplasmic. Thus, the question why $r_{2,4}$ does not restrict unmodified progeny DNA is still unresolved. We report here that the phage specifies a protein which prevents the action of $r_{2,4}$. The anti-$r_{2,4}$ activity specified by the modified parental genome prevents restriction of the unmodified progeny DNA by $r_{2,4}$.

Of the two restriction activities of the host, coded by the *rgl* system, r_6 seems more readily and rapidly inactivable, since it is inhibited by phage infection even in the absence of expression of the phage genome[13,14]. Table 1 shows that the inactivation of $r_{2,4}$, in contrast to r_6, requires expression of the phage genome. Infection of $r_6^+r_{2,4}^-$ cells inactivates the r_6 activity in the presence or absence of primary phage expression. In $r_{2,4}^+$

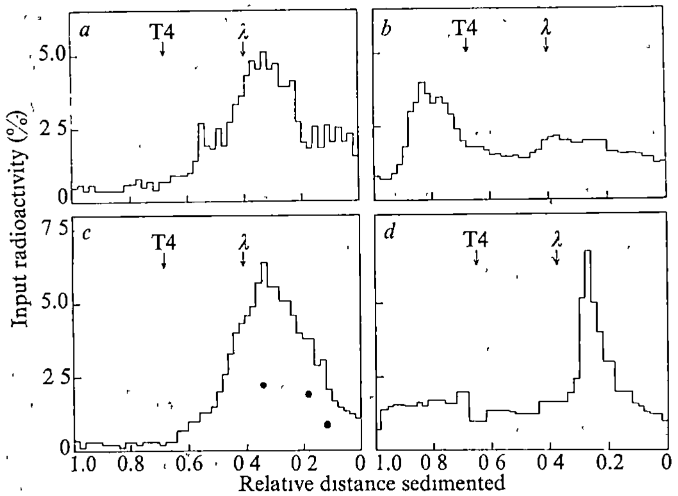

Fig. 1 Demonstration of a protein inhibiting the cleavage of superinfecting glu$^-$ T4 DNA. *rgl*$^+$ exo V$^-$ (W3110 recB$^-$) cells infected with T4 *imm*$^-$ *s*$^-$ phage (which permit less leakage of *imm* expression late in infection[18]) in the presence or absence of chloramphenicol, were superinfected with ^{32}P-glu$^-$ T4 (T4α' gt^{am8} βgt^{am10}.K rgl$^-$ su$^-$) after 10 min. Five minutes after superinfection, native DNA molecules were extracted[20] and analysed in neutral sucrose gradients. The same preparations, denatured by adding sodium hydroxide to 0.4 N, were also analysed in alkaline sucrose gradients[16]. Centrifugation was for 110 min at 147,000*g* at 20 °C. ^{3}H-λC$_{1857}$susS$_7$ particles[19] were added to the infected *E. coli* during lysis of spheroplasts by detergent, to serve as a marker. The calculated[21] position of T4 DNA was confirmed on several occasions. *a*, Chloramphenicol absent, alkaline gradient; *b*, chloramphenicol absent, neutral gradient; *c*, chloramphenicol present, alkaline gradient; *d*, chloramphenicol present, neutral gradient.

cells the primary infecting *imm*$^-$ phage prevents the breakdown of superinfecting glu$^-$ phage, only when primary phage expression takes place. In these cells, when primary phage expression is prevented by chloramphenicol or ultraviolet irradiation (UV), of the primary infecting virions the superinfecting glu$^-$ T4 is broken down. This implies that the primary infecting phage codes for a protein which prevents the breakdown of glu$^-$ T4 DNA. We have shown that the restriction of unmodified T4 *in vivo* takes place in two steps[16]. The first step caused by the restriction endonuclease, which we call cleavage, results in double-stranded breaks in the unmodified genome. The large trichloroacetic acid (TCA)-insoluble double-stranded pieces are further degraded by exonuclease (exo) V (the rec BC enzyme) to small acid-soluble and insoluble fragments[13,16]. We call this second step DNA breakdown.

T4 makes a protein which prevents exo V action[17,18]. In the experiment described above we measured only DNA breakdown. The inhibition of breakdown by the primary phage-coded proteins may reflect either inhibition of exo V or inhibition of cleavage by restriction enzymes or both. To see whether cleavage was inhibited, we measured the cleavage reaction alone in exo V$^-$ hosts. *E. coli rgl*$^+$ infected with T4 *imm*$^-$*s*$^-$ phage, permits superinfection by glu$^-$ T4 phage without DNA breakdown[13]. Figure 1 shows the fate of this superinfecting phage DNA. When sedimented on an alkaline sucrose gradient the strands are shown to be fragmented to 1/4–1/20 their original length, as might be expected for restriction cleavage of glu$^-$ DNA by *rgl* (Fig. 1*a*). But this same DNA, when native, sediments mainly as concatemers (Fig. 1*b*). This, however, is the result expected for glu$^-$ DNA on a phenotypically *rgl*$^-$ host, that is if $r_{2,4}$ were inactivated. When this

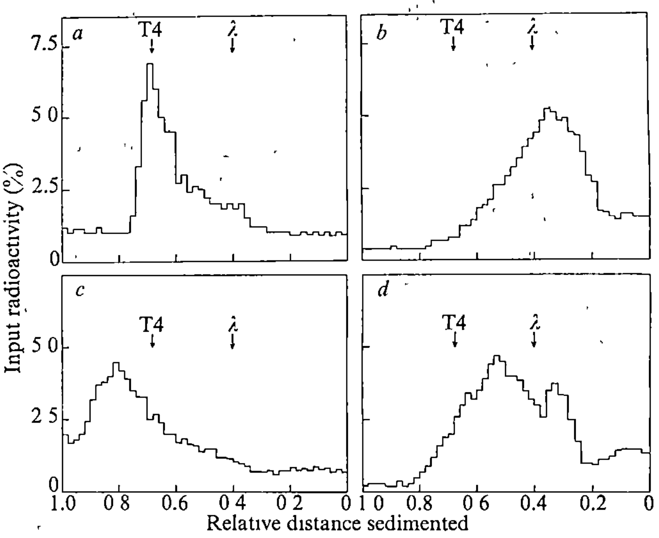

Fig. 2 Demonstration of a chloramphenicol-sensitive nicking enzyme in T4 *imm*$^-$*s*$^-$ infected cells. Ten minutes after primary infection with T4 *imm*$^-$*s*$^-$ phage the cells were superinfected with the appropriate ^{32}P-phage. Five minutes after superinfection DNA was extracted and analysed for size distribution in native and denatured conditions. Experimental details are described in legend for Fig. 1. *a*, *E. coli* K *rgl*$^-$ cells superinfected with glu$^-$T4, chloramphenicol present, alkaline gradient; *b*, *E. coli* K *rgl*$^-$ cells superinfected with glu$^-$T4, chloramphenicol absent, alkaline gradient; *c*, *E. coli* K *rgl*$^-$ cells superinfected with glu$^-$T4, chloramphenicol absent, neutral gradient; *d*, *E. coli* K *rgl*$^+$ exo V$^-$ cells, superinfected with T4 wild-type phage, chloramphenicol absent, alkaline gradient.

Table 1 Effect of primary infecting phage-coded function(s) in the breakdown of superinfecting glu$^-$ T4

Bacterial strains	Chloramphenicol	Primary phage	Superinfecting phage	% DNA breakdown
$r_6^-r_{2,4}^-$	−	T4glu$^-$	—	4
	−	T4*imm*$^-$	T4glu$^-$	3
	+	T4*imm*$^-$	T4glu$^-$	3
	−	UV.T4	T4glu$^-$	14
$r_6^+r_{2,4}^-$	−	T4glu$^-$	—	28
	−	T4*imm*$^-$	T4glu$^-$	4
	+	T4*imm*$^-$	T4glu$^-$	3
	−	UV.T4	T4glu$^-$	16
$r_6^-r_{2,4}^+$	−	T4glu$^-$	—	30
	−	T4*imm*$^-$	T4glu$^-$	7
	+	T4*imm*$^-$	T4glu$^-$	28
	−	UV.T4	T4glu$^-$	43
$r_6^+r_{2,4}^+$	−	T4glu$^-$	—	35
	−	T4*imm*$^-$	T4glu$^-$	5
	+	T4*imm*$^-$	T4glu$^-$	36
	−	UV.T4	T4glu$^-$	35

Phage and bacterial strains, culture conditions and other techniques used in this study are described previously[16]. Log phage cells (5×10^8 cells per ml) were infected with 3–4 T4 *imm*$^-$ phage per bacterium. 2–3 ^{32}P-glu$^-$ T4 was added 5 min after primary infection. DNA breakdown was determined as trichloroacetic acid (TCA)-soluble fraction of DNA, 5 min after superinfection. Chloramphenicol (100 μg ml^{-1}), when required was added 3 min before primary infection. The data given are not corrected for adsorption, which was 85–95%.

experiment is repeated in the presence of chloramphenicol not only are nicks observed in the isolated strands (Fig. 1*c*) but double-stranded cleavage (Fig. 1*d*) is observed, as expected for glu$^-$ DNA in an *rgl*$^+$ host. Thus it seems that the cleavage activity of $r_{2,4}$ on double-stranded DNA is inhibited by a primary infecting phage-coded protein, the synthesis of which is inhibited by chloramphenicol.

To be certain that nicking in Fig. 1*c* was caused by $r_{2,4}$, we repeated the experiment using an *rgl*$^-$($r_6^-r_{2,4}^-$) host cell. *rgl*$^-$ cells infected with T4 *imm*$^-$*s*$^-$ in the presence of chloramphenicol were superinfected with glu$^-$ T4. Single strands of DNA sediment mainly as whole strands (Fig. 2*a*), but in the absence of chloramphenicol the superinfecting glu$^-$ DNA is nicked (Fig. 2*b*). Thus there are two different nicking (endonuclease) activities in the cell at different times. The first, the host *rgl* system, nicks complementary DNA strands in close proximity leading to DNA cleavage. This cleavage activity of the host *rgl* system can be demonstrated even in the presence of chloramphenicol in *rgl*$^+$ cells but not in *rgl*$^-$ cells in the absence or presence of chloramphenicol (compare Figs 1*d* and 2*c*). The host cleavage activity is inhibited by a phage-coded protein which appears after primary infection and is not made in the presence of chloramphenicol (compare Fig. 1*b* and *d*). The second, a phage-coded enzyme, produces single-stranded nicks but does not cleave double-stranded DNA. We think that this enzyme is probably the phage endonuclease demonstrated *in vitro* by Altman and Meselson[22]. Thus, in the absence of chloramphenicol, when *rgl* cleavage activity is inhibited, nicking activity is still apparent (compare Fig. 1*a* and *c*). This activity is independent of the *rgl* system and requires phage protein synthesis (compare Fig. 2*a* and *b*). The nicking activity is also not greatly affected by glucosylation since both glu$^+$ and glu$^-$ DNA are nicked in these conditions (compare Figs 2*d* and 1*a*). In any case, data in Figs 1 and 2 show that a protein is made, after primary phage infection, which prevents initial cleavage of superinfecting glu$^-$ DNA by $r_{2,4}$. We call this protein anti-$r_{2,4}$.

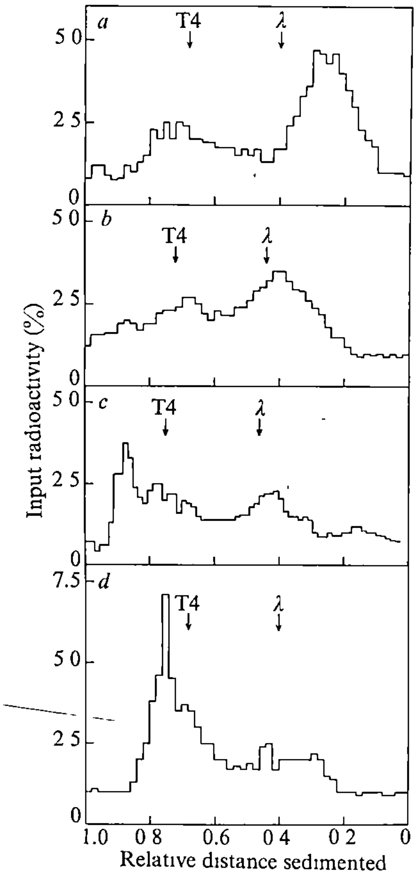

Fig. 3 Time of appearance of anti-$r_{2,4}$.*rgl*$^+$ exo V$^-$ cells infected with T4 *imm*$^-$*s*$^-$ phage were superinfected with ^{32}P-glu$^-$T4 at various times. Native DNA was analysed 5 min after superinfection. *a*, *b*, *c* and *d* were superinfected at 1, 2, 5 and 7 min after primary infection respectively.

To establish the time of appearance of the anti-$r_{2,4}$ activity we infected *E. coli rgl*$^+$ with T4 *imm*$^-$*s*$^-$ phage and superinfected with glu$^-$ T4 at various times after primary infection. Figure 3 shows that large molecules, of mature T4 DNA size or larger, accumulate as the time of superinfection is delayed. This anti-$r_{2,4}$ activity appears quite soon after infection. The exact time of its appearance is difficult to establish in these experiments, however, since it is still being synthesised between the time of superinfection and completion of cleavage of the superinfecting glu$^-$ phage DNA. Therefore, the 1-min point has probably allowed more than 1 min of expression by the primary phage. By 7 min after infection, when unmodified progeny DNA synthesis is expected to start, $r_{2,4}$ activity has disappeared.

An intriguing problem in the restriction system of glu$^-$ T4 is the successful replication of intracellular unmodified genomes in contrast to the complete restriction of entering parental unmodified genomes. The demonstration of the anti-$r_{2,4}$ activity gives a satisfactory explanation for the resistance of unmodified progeny genomes to restriction. This does not seem to be so in the case of phage λ. The analogous experiment, infection of restricting bacteria ($r_{\rm K}^+$) by modified λ in the presence of an inhibitor of S-adenosylmethionine (SAM) production, leads to the production of normal unmodified phage[23]. SAM (unlike UDPG in the case of T4) is, however, needed for restriction as well as modification[8]. When a similar experiment was repeated using a P1 lysogen, the λ progeny were restricted as SAM is needed for P1 modification but not for restriction[23]. This experiment also shows that no inhibitor of the P1 restriction enzyme is made in this case. Nevertheless it is possible that complementation of modified λ by superinfecting unmodified λ in a non-permissive host[24] may involve production of anti-$r_{\rm K}$. Direct measurement *in vivo*, of cleavage activity by $r_{\rm K}$ (ref. 16) in these conditions should decide this question. It is probable, however, that complementation by cleaved unmodified fragments is enhanced by the inactivation of exo V[25] by the gamma gene-product from the primary infecting modified λ phage[26].

This research was supported by grants from National Institutes of Health and the US National Science Foundation. E.B.G. is a career development awardee of the National Institutes of Health.

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matters arising

Tornado forum

RECENTLY the proposal was made that motor traffic has contributed significantly to the sixfold increase in incidence of tornadoes in the USA in the past forty years.[1] The authors saw the vorticity introduced into the atmosphere by traffic driving on the right as favouring the generation of cyclonic tornadoes, and they were encouraged in this hypothesis by an increase in the proportion of cyclonic to anticyclonic tornadoes, and also by the low incidence of tornadoes reported on Saturdays, when traffic would be lower and more unidirectional (away from large cities).

The suggestion has provoked many responses and to publish them all would have occupied more space than we could allot. So we print below abridged versions of some of the communications received, followed by a reply from the authors.

[1] Isaacs, J D, Stork, J W., Goldstein, D B., and Wick, G. L, *Nature*, 253, 254–255 (1975).

A MOTOR vehicle or any other projectile in air produces vortices in its wake, but no net vorticity. If there are anticyclonic vortices along edges of the highway, as shown in Fig. 1 of Isaacs *et al.*, their vorticity must be balanced by cyclonic vorticity in the median strip.

The width of a highway is small compared to a cloud, and a significant updraft entraining the vortices produced by motor vehicles would realise no net contribution from that source. The main atmospheric phenomena produced by cars are turbulence, and gaseous and particulate combustion products along the highway, dissipating and diffusing downwind.

The low incidence of tornadoes on Saturdays perhaps reflects some unfortunate vagaries in reporting. Or, conceivably, changes in the atmospheric radiation budget and aerosol content induced by weekend factory closings and lowered air pollution produce changes in static stability and cloud particle development.

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WE believe that the mechanism for vorticity generation and accumulation in the atmosphere proposed by Isaacs *et al.* is based on misconceptions about the nature of vorticity. The atmosphere contains background vorticity arising from the rotation of the Earth and relative vorticity from weather disturbances and boundary effects, including moving vehicles. In fact the background vorticity can be ignored to high accuracy in the dynamics of flow around vehicles, since Coriolis effects are minute. The following argument depends, however, mainly on kinematical results, which are exact.

We begin by considering the generation of vorticity by a single moving vehicle. Absolute vorticity can be generated in a homogeneous fluid only at boundary surfaces moving relative to the fluid[1], and so is generated at the sides, top and bottom of the vehicle, at its wheels and at the ground in its neighbourhood. Once generated it is advected with the air stream into the vehicle's wake. The vortex filaments of the resulting relative vorticity vector field ω must either form closed loops or terminate at a boundary rotating relative to the Earth; and on this basis we can distinguish two constituents in the relative vorticity generated at a moving vehicle:

(1) When the vehicle moves in a straight line, closed vortex loops are shed continuously into its wake. These profoundly affect local levels of vorticity close behind the vehicle, but have no effect whatever either on the circulation round a contour enclosing the wake, or on the bulk integral

$$\int_V \omega \mathrm{d}V$$

over a volume V including the wake and the neighbourhood of the vehicle, because each vortex filament is closed. (None of these facts is changed by the presence within V of more vehicles whether driving on the right or on the left.)

(2) Vorticity having a net vertical component is generated when a vehicle turns, at a rate proportional to minus the rate of change of angular velocity of the vehicle about the vertical; see equation (1). There is an additional net horizontal component associated with the rotation of the wheels, which need not concern us further.

It is immediately clear that randomly varying vehicle movements can have no systematic gross effect over a large area as claimed by Isaacs *et al.* The vehicles could, however, produce localised concentrations of vorticity (changing sign over length scales of a few metres) the significance of which will depend very much on persistence and hence on interdiffusion rates. We shall discuss this question further elsewhere, but remark here that order-of-magnitude estimates based on plausible levels of turbulence suggest persistence times of minutes rather than hours, and vortices of dust devil rather than tornado scale.

If, as an opposite extreme, a large number of vehicles were to be driven simultaneously in fast clockwise circles, there would indeed temporarily be a cyclonic (that is, anticlockwise) contribution to the bulk vorticity integral. But this can be shown to be associated entirely with the air below vehicle rooftop level or, more precisely, below a plane surface s lying just above all the vehicles. When summed over the vast bulk of the atmosphere lying above s, the total component of vorticity normal to s caused by the vehicles is, and must remain, exactly zero (see equation (2) below). This is a consequence of the simple fact that each vehicle-generated vortex filament passing upward through s must go back down through s at another point, possibly terminating on a moving surface such as the underside of the same vehicle.

In summary, neither mechanism (1) nor (2) can plausibly be connected with the generation of tornadoes, which takes place high above the ground and which involves air motions coherent over length scales of kilometres.

Isaacs *et al.* stated that cyclonic vorticity "is generated by the torque between the two opposing streams of traffic", and this led to their view that it is better to drive on the left than the right of the road. Unfortunately torque does not determine the generation of vorticity, either locally or globally. Although doubling the separation of two traffic streams may approximately double the torque (to say nothing of the far larger contribution from unidirectional traffic on widely-separated highways) it has absolutely no effect on the net generation of vorticity. The vorticity due to the traffic streams is, to an excellent approximation, the sum of the contributions produced at the surface of each vehicle, locally redistributed by advection and diffusion; and any effects on the bulk vorticity integral have nothing to do with the separation of traffic streams, nor with left as against right hand drive.

The basic mathematical fact underlying our discussion is the solenoidal property of ω: since ω is the curl of a velocity field, $\nabla.\omega$ is identically zero. It follows that

$$\int_V \omega_i dV = \int_V \frac{\partial}{\partial x_j}\left(x_i\omega_j\right)dV$$
$$= \int_S x_i(\omega.\mathrm{n})dS$$

where V is a suitable piece of the atmosphere, partially bounded by the surface S, comprising the ground and the surfaces of stationary and moving objects (vehicles, their wheels, aeroplanes) which are envisaged as the source of the contribution ω to the relative vorticity, itself supposed confined within V. From this identity it may readily be shown that

$$\int_V \omega dV = -2\sum \Omega_r(t)V_r \qquad (1)$$

where $\Omega_r(t)$ is the angular velocity of the rth moving body, and V_r its volume. We here use the fact, fundamental to the vorticity generation mechanism, that the no slip condition[1] applies at all parts of the surface S.

Further, if all the moving objects lie below a plane surface s then, if ω_3 is the component of ω normal to s and v is the part of V above s,

$$\int_S \omega_3 dS = -\int_V \nabla.\omega dV = 0$$

Hence, by integration with respect to x_3,

$$\int_V \omega_3 dV = 0 \qquad (2)$$

The results (1) and (2) are both exact, even for an atmosphere in which the motion is baroclinic, compressible, and turbulent.

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ISAACS *et al.* assert that the opposing streams of traffic on a road induce a net cyclonic vorticity in the atmosphere. Because vorticity diffuses exponentially slowly at large distances from a source, however, this is not so.

If the opposing traffic streams are modelled by infinite plates a distance D apart, then the resulting mean flow field at any finite time after the motion begins is of the form shown in Fig. 1. The detailed behaviour of the mean velocity profile near the plates is unimportant. Although the motion near the plates is turbulent in general, any far-field disturbance must propagate normal to the plates by the action of molecular diffusion, and so the mean velocity decays exponentially as $y \to \infty$. Thus, integrating the mean vorticity $\partial U/\partial y$ with respect to y, we find that the total vorticity per unit distance along the plates in the region $|y| > D/2$ is finite and equal to $2U_0$, whereas the total vorticity in $|y| < D/2$ is equal to $-2U_0$, where U_0 is the speed of each plate. Thus the cyclonic vorticity in the region between the plates is just balanced by the anticyclonic vorticity outside the plates. Alternatively, we see that the net circulation around a closed contour (as shown by the dotted line in Fig. 1) goes to zero as the y dimension of the contour increases. This is because the cyclonic torque induced by the plates is opposed by the inertia of the fluid at infinity, which is at rest.

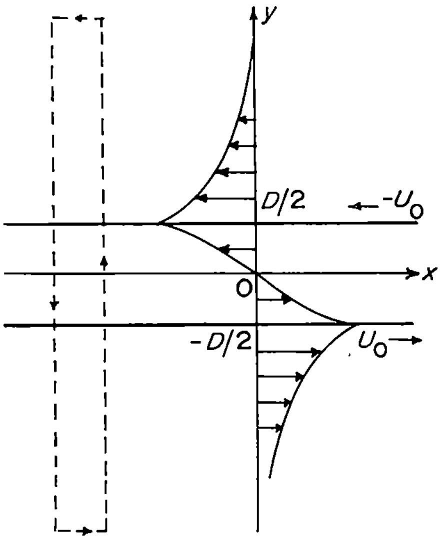

Fig. 1 Mean velocity $U(y)$ associated with the motion of two infinite plates ($y = D/2$ and $-D/2$) travelling at speed U_0 in opposite directions.

This result implies that although cyclonic vorticity is generated between the streams of traffic, there is no net generation of vorticity, because an equal amount of anticyclonic vorticity is also induced. It would seem therefore that, according to the hypothesis of Isaacs *et al.*, either both cyclonic and anticyclonic tornadoes ought to be equally probable, or no tornadoes ought to be generated, depending on whether the air is not, or is mixed horizontally in the process of tornado generation.

On the other hand, Isaacs *et al.* do give some strong circumstantial evidence for the connection between cyclonic tornadoes and traffic-induced vorticity. Their hypothesis can perhaps still be retained if the second ingredient involved in the generation of tornadoes, namely convection, is included. Any vorticity induced by traffic is initially confined to a layer a couple of metres deep near the ground. To produce a tornado, this vorticity must be preferentially advected vertically. Such motions are found in convection, which is an anisotropic process where horizontal mixing is suppressed[1]. Moreover, road pavement in general absorbs more solar radiation than does the surrounding terrain and so the road is expected to be a stronger source of convection than the surrounding terrain. (The cars themselves also act as a local heat source.) Convection therefore ought to occur preferentially from the road. Because the cyclonic vorticity (of mean strength $2U_0/D$) is concentrated over the road while the anticyclonic vorticity is diffused on each side of the road, it would appear that the convection leads to the preferential advection of cyclonic vorticity into the troposphere, as required by Isaacs *et al.* That is, cyclonic tornadoes can be produced because roads are sources of both cyclonic vorticity and convection.

This work was done in the Department of Mathematics, University of British Columbia, and was supported in part by the NRC.

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[1] Manton, M. J., *Aust. J. Phys.*, 27, 495 (1974)

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A CALCULATION is indicated, though not shown, by Isaacs *et al.*, purporting to prove that motor vehicle traffic contributes significantly to the atmospheric vorticity over the USA and thereby leads to a more homogeneously cyclonic troposphere and consequently to changes in the broad scale spatial and temporal distribution and frequency of tornadoes. In the following I have attempted to repeat the calculation, with results substantially different from those of the authors.

A typical round trip is taken to be a straight line of length L, a straight line return and a U-turn at each end, with a separation between lanes of width d. If the average force exerted backwards by the wheels on the road is F, then the net anticyclonic angular momentum added to the earth, located at the centroid of the path, is $2FLd/V$, where V is the automobile speed, with nominally equal contributions coming from the straight legs and the U-turns. We assume the force F to be the air drag of the vehicle, given by

$$F = \rho a C_D V^2/2 \qquad (1)$$

where ρ is the air density, a is the cross-sectional area of the vehicle, and C_D is the drag coefficient, taken to be 0.5.

Since the speed while making the U-turns is likely to be much less than that on the straight legs, we ignore those contributions, and the average torque produced by the vehicle is then

$$T = Fd = \rho a C_D V^2 d/2 \qquad (2)$$

Isaacs *et al.* assume that 2×10^6 automobiles are on the road in the USA at any time, and that the contributions to torque from autos and trucks are equal. Using these figures, and assuming a mean cross-sectional area of 3 m^2, $V = 20$ m s^{-1}, the lane spacing $d = 20$ m, and $\rho = 1.3$ kg m^{-3}, we obtain a net torque production of 3.2×10^{10} kg m^2 s^{-2}. In this calculation, the difference in direction between the torques produced in different parts of the USA is ignored, which amounts to assuming the cosine of angles smaller than ~20° to be unity.

The angular momentum, M, of a rotating body is the second radial moment of its vorticity

$$M = \int \zeta r^2 \mathrm{d}m$$

where ζ is the vertical component of vorticity and dm is the mass differential. Over the USA during most seasons, the mean vorticity of the atmosphere is close to the vertical component of the Earth's vorticity, $f \simeq 10^{-4}$ s^{-1}. Thus the angular momentum of the atmosphere of the USA is roughly

$$M = (p_0/g)Afr^2/2,$$

where p_0 is the mean surface pressure, g is the acceleration of gravity, A is the area of the USA, and r is the mean radius, corresponding to the moment of inertia of the USA, which we estimate to be 2,000 km. Taking p_0/g to be 10^4 kg m^{-2} and $A = 8\times10^{12}$ m^2, we obtain $M = 1.6\times10^{25}$ kg m^2 s^{-1}. Thus it would seem that the motor vehicle torque would have to continue for 10^{14} s, about 3 Myr, to show a noticeable effect. The natural turnover time of angular momentum in the atmosphere is of order days or weeks, however, so that the effects suggested by Isaacs *et al.* seem very unlikely.

About the largest effect that could conceivably be produced in the way the authors envisage would occur if all the vehicles in the USA drove in the same direction along highways close to the USA coastlines and borders. In this case the effect would be increased by a factor of 10^5, but would still be too small to be noticeable.

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THERE is a Saturday anomaly in the reporting procedures that affects the final numbers in the paper of Isaacs *et al.* The major source of information about tornadoes in the USA since 1953–54 has been press clippings from out-state newspapers. This drastic change in data-gathering techniques can best be seen in the figure on p. 35 of ref. 1. The average annual totals jumped from ~ 280 yr^{-1} to 640 yr^{-1} beginning in 1954. In Missouri the use of press clipping information coincided with a change in the average annual total reports from 13 yr^{-1} to 33 yr^{-1}. One might conclude, therefore, that in Missouri almost two-thirds of the total reports are strongly dependent on press clipping information. For the entire USA it would seem that more than half of the total number of reports depend to some extent on press clippings.

In general, these additional tornado reports generated by press clipping information tend to be the smaller, short lived mini-tornadoes in largely rural areas. The small amount of property damage and infrequent personal injuries does not make them newsworthy items for the large metropolitan dailies. These storm reports are frequently covered only by local weeklies and the smaller out-state 'daily' papers. It is in these 'dailies' that the anomaly on Saturdays has its origins.

A substantial percentage of out-state daily papers do not publish on Sundays, and a surprising number do not publish on both Saturday and Sunday. In Missouri, for example, 45 of the 61 daily papers do not publish on Sunday and 24 of 61 do not publish on both Saturday and Sunday. Thus, the events of Saturday are frequently old news by the time these dailies go to press on Monday. The minor tornado activity that occurs on Saturday is not nearly as intensively covered as a similar occurrence on any other day of the week. This discontinuity in the basic source of information for tornado reports could easily account for a decrease in Saturday totals of ~ 10–15%. When up to two-thirds of the basic information sources for more than half of the usual total reports cut back to 25–50% efficiency for Saturday events, we end up with an anomaly.

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[1] Court, A., *Tornado Incidence Maps, ESSA Tech. Memo ERLTM—NSSL*, 49 (1970).

THE evidence given by Isaacs *et al.* for their discovery is that, first, "the annual centre of tornado intensity weighted by area . . . has increasingly occupied the eastern portion of the continent", and second that of those few tornadoes for which rotation sense was reported, during the past 12 yr (1962–1973) 8.1% were anticyclonic compared with only 36.4% in the preceding 12 yr; and third, especially, that only 1,868 of 15,234 tornadoes during 22 yr occurred on Saturdays.

Tornadoes are reported by people according to damage done, and as the US population expanded westwards so did tornado reports. Since 1925, the apparent centre of tornado incidence has moved generally south-westwards into eastern Kansas (by 1925), to central Kansas (by 1951), and to central Oklahoma (by 1963). Oklahoma, now the region of greatest incidence, was shown as tornado deficient on earlier maps because it was the last Plains state to be settled[1].

Rotation sense is given for too few tornadoes to be of significance; the big surprise is that any anticyclonic tornadoes whatever are reported. "Motor vehicles are the only man-made source of non-random vorticity we know", the authors say. Perhaps they have never seen two railway trains pass at full speed, or noted the action of exhaust fans and rooftop ventilators.

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[1] Court, A., *Tornado Incidence Maps, ESSA Tech. Memo. ERLTM-NSSL*, 49 (1970).

ISAACS*, STORK AND WICK* REPLY—Opposing streams of traffic in the USA exert anticyclonic torques on the solid Earth and, from action and reaction, compensating cyclonic torques on the atmosphere. These torques tend to slow down the Earth's rotation and to increase the cyclonic rotation of the local atmosphere in conservation of angular momentum.

We agree with Kessler[1], Morton *et al.*[2] and Manton[3] that there is zero net vorticity in the total atmosphere caused by traffic. Our original discussion was based on vorticity rather than angular momentum. We were in error in not recognising that a discussion of the process from a consideration of vorticity is much less direct than from a consideration of angular momentum. Angular momentum is a more fundamental quantity in the formation of strongly rotating storms.

There can be little doubt that US automotive traffic introduces cyclonic angular momentum into the local atmosphere, which can participate in the formation of tornadoes. To illustrate how this occurs, consider this example: if you set fluid in a bowl into circulation by periodically immersing your hands and moving them past one another in straight lines always in the same relative direction but in any orientation and location, the fluid will gain angular momentum. Yet for this same fluid the no-slip condition at the boundaries requires zero net vorticity. The boundary

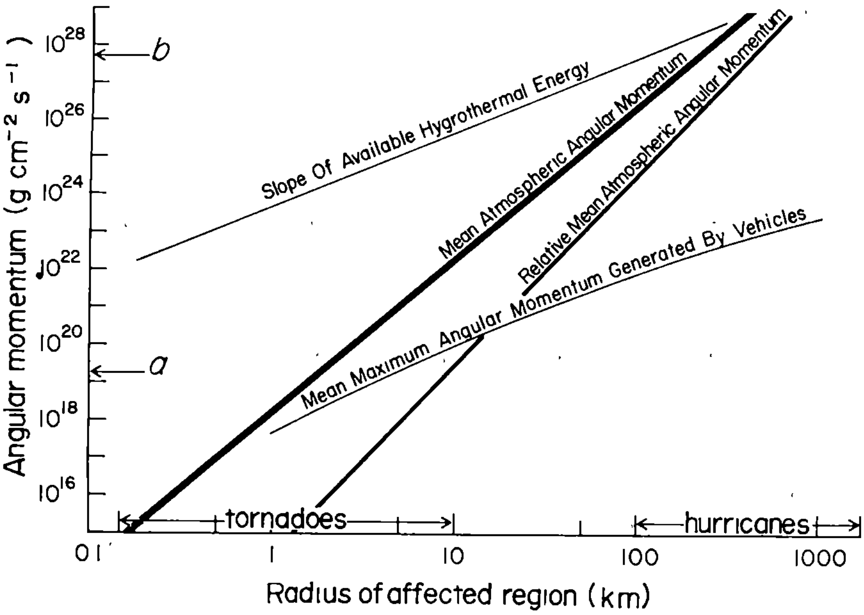

Fig. 1 Atmospheric angular momentum as a function of the affected area and anthropogenic input. The mean atmospheric angular momentum contained in a mass of air is that due to its planetary rotation around its central axis and is involved in radial convergence. It scales as the fourth power of the radius of the affected area. The mean relative atmospheric angular momentum is that involved in linear convergence over the dimensions on the abscissa, and scales as the fifth power of the radius. (Both are at $\sim$45° latitude) The angular momentum generated by the vehicles is based on the area, the residence time of air over the region (10^5–10^6 s) and the maximum density of traffic within such an area. The dimensions involved in the convection of a tornado or hurricane are indicated on the abscissa. The points labelled *a* and *b* indicate the angular momentum of a single giant tornado and of a hurricane, respectively. For a similar atmosphere and similar equilibrium altitude, the hygrothermal energy available to convective storms scales as the square of the radius of the affected region. (The line is drawn with an arbitrary ordinate intercept.)

vorticity does not preclude the formation of a vortex when the fluid is allowed to flow through an orifice in the bottom of the bowl. The important points are that angular momentum can be introduced by linear motions in this way, and that it can participate in a vortex while the net vorticity remains zero.

The apparent paradox of automobile-generated angular momentum with zero vorticity is probably resolved through several mechanisms in the atmosphere. As in the example of the bowl, the boundary vorticity in the atmosphere may propagate and act outside the area of convergence, as we originally proposed. The resolution may not be unrelated to our recent observation alongside a highway bearing heavy traffic that anticyclonic filaments of small-scale rotation are embedded in large-scale cyclonic rotation

At any rate, our calculations of the torque and resulting angular momentum agree with those of Lilly[4], but are more conservative by a factor of about eight. We do not consider, as he did, that the torque on the atmosphere be applied to the entire air mass over the USA as though the atmosphere were a solid body. Rather this torque must exert its influence locally, and in areas consonant with those involved in the convective processes of a tornadic storm. Furthermore, if variations in local vehicle concentrations of two orders of magnitude are taken into account, our calculations show that vehicle-produced torque, over reasonable periods of time, can produce angular momentum of the same order or greater than the natural background (see Fig. 1) The angular momentum introduced annually by vehicles is sufficient to account for that present in $\sim 10^5$ giant tornadoes. (A total of $\sim$ 600 including all sizes are actually reported per year.)

Court's point[5] that the centre of tornado occurrence has moved westward makes it even more impressive that the centre of tornado intensity, which is what we reported, has increasingly occupied the eastern sections of the USA. The obvious inference here is that the more intense tornadoes have been increasingly found in the east in support of our original thesis.

We agree that too few observations of the rotational sense of tornadoes have been reported to make these data highly significant. A χ^2 test based on these data shows, however, a statistically significant shift from anticyclonic to cyclonic tornadoes over the 24-yr period, and thus these data support our hypothesis. The fact that anticyclonic tornadoes exist at all is important in showing that local effects other than planetary rotation can be significant for the formation of tornadoes. One of these local effects could be vehicle-generated cyclonic angular momentum.

Darkow[6] raises an interesting point in attributing the weekly periodicity of the tornado data to periodicity in newspaper reporting. The possibility of reporting peculiarities being the cause has also concerned us. Before our initial publication, one of us contacted the Administrator of the National Oceanic and Atmospheric Administration, who was certain that weekend reporting was adequate or even intensified because of increased exposure to more people at weekends. In addition, since the data on the tornado tape we acquired from the National Severe Storms Forecast Center (NSSFC) are listed by source, we were able to separate out those that were identified as press clipping data (33%). The remaining data showed an even stronger weekly periodicity than reported for the total. These remaining data are considered to be the most reliable data by NSSFC.

As a further check we had plotted tornado injuries, damage, tornado days, path area and sums of *f*-scale numbers by day of week. We thought that tornadoes of sufficient significance to require the reporting of these parameters would not be subject to such reporting vagaries as might affect the reporting of lesser tornadoes. In every case Saturday was low, thus increasing our confidence in the validity of the findings.

Weekend changes in the production of factory wastes could conceivably influence the static stability and cloud-particle development as suggested by Kessler[1]. It is difficult, however, to see why this phenomenon would have its effect only on Saturday and not on Sunday. Presumably a factory that is closed on Saturday will remain so on most of Sunday. As a further check, we have obtained a tape of thunderstorm data from NSSFC and analysed it for weekly periodicity. We found none. Thus it seems that the periodicity in tornado occurrence is not dependent on convective storm periodicity but more probably on the periodicity of input of that unique and essential ingredient of tornadoes–angular momentum

Referring again to Fig. 1, the planetary angular momentum scales by the fourth or fifth power of the radius of the area involved in convection, depending on the type of convergence, while energy scales only as the second power of the radius. Thus a strong argument can be made that, if energy limits the population of strongly rotating convective storms of the magnitude of hurricanes (which seems to be the case), storms of the magnitude of tornadoes are comparatively deficient in planetary angular momentum, as related to energy by at least three orders of magnitude. The fact that most thunderstorms do not involve tornadoes seems to support this statement. This argues that angular momentum, rather than energy, limits the population of tornadoes, and that any input of cyclonic angular momentum increases their incidence, as we have proposed.

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[1] Kessler, E., *Nature*, 260, 457 (1976).
[2] Morton, B. R., Smith, R. K., and McIntyre, M. E., *Nature*, 260, 457–458 (1976).
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[4] Lilly, D. K., *Nature*, 260, 458–459 (1976).
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[6] Darkow, G. L., *Nature*, 260, 459 (1976).

Identification of averaged auditory evoked potentials in man

SAYERS *et al.*[1,2], in their work on the Fourier analysis of averaged auditory evoked potentials (AEP) have suggested that suppositions for arithmetic averaging are violated, since there are no differences between the mean powers in the pre- and post-stimulus averaged potentials. Their results contradict the fact that the amplitudes of AEP seem much more pronounced in comparison with averaged spontaneous EEG activity.

Fourier analyses of averaged potentials (Fig. 1) show that the forms of both amplitude and phase spectra in the post-stimulus period, where the AEP can be seen, differ significantly from those in the pre-stimulus period, where no response occurs. The maximum in the amplitude spectra of post-stimulus averaged signals (the region of the AEP) with lower sound intensities diminishes and shifts to lower frequencies. In the pre-stimulus regions the amplitude and phase spectra always have irregular forms and lower amplitudes. Therefore, both the amplitude and the phase spectra are of importance in the objective detection of AEPs by Fourier analysis.

Further, the mean power $(P_{\bar{x}})$ of the post-stimulus averaged signal is higher than in a corresponding length of the pre-stimulus period (Table 1). This behaviour can be observed down to the hearing threshold in cases of low spontaneous EEG activity.

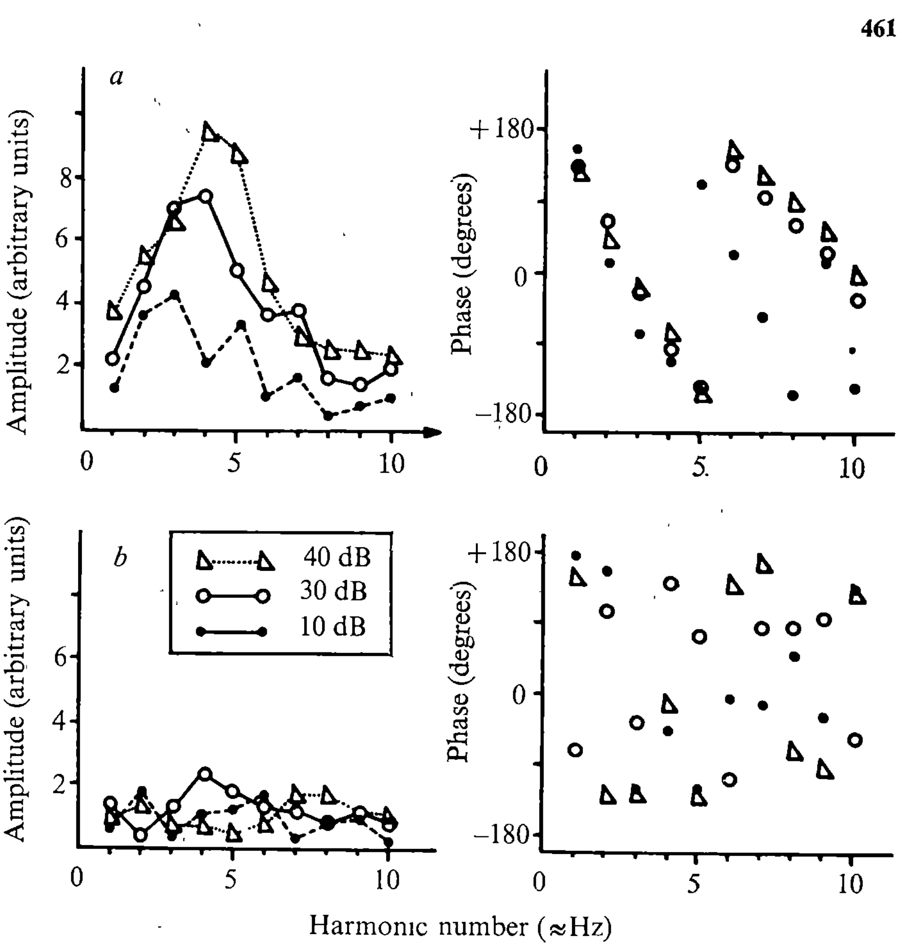

Fig. 1 Normal hearing female adult, binaural stimulation with 1-kHz tone bursts of 300-ms duration and 8-ms rise-fall time, interstimulus interval 3.2 s, $n = 50$ averaging cycles. Parameter: sensation level; frequency interval between harmonics 0.98 Hz; connecting lines illustrate typical behaviour. *a*, Amplitude and phase spectra of averaged responses (post-stimulus periods, 0–1,023 ms after onset of stimulation). *b*, Amplitude and phase spectra of averaged spontaneous activity (pre-stimulus periods, 1,800–777 ms before stimulation). △, 40 dB; ○, 30 dB; ●, 10 dB.

To show that single AEPs consist of an additive contribution to the spontaneous EEG we evaluated the averaged power spectrum (by means of the sum of power spectra of the single EEG responses) for both pre- and post-stimulus periods. The mean power $(\bar{P}_{x_i})$ of pre- or post-stimulus electroencephalic activity can be calculated using this averaged power spectrum. The observed increase of $\bar{P}_{x_i}$ in the post-stimulus period (Table 1) is in agreement with the assumption of an additive superposition of the single AEPs to the spontaneous EEG (see ref. 3).

Averaging the squared single EEG responses, $x_i^2(t)$, gives the same result. The evaluated mean square values

$$\overline{x^2(t)} = (1/n)\sum_{i=1}^{n} x_i^2(t)$$

are proportional to the mean power of the EEG. Because of an additive contribution of single responses to the spontaneous EEG an increase of the mean power in the region of the AEP is detectable down to 20 dB.

Using the mean square values $\overline{x^2(t)}$ it is possible to calculate the standard deviation $s(t)$ of the AEPs. In this way the statistical significance of registrated AEPs can be evaluated without using the Fourier method[2] with its more complex problems of utilisation.

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Table 1 Mean power of averaged potentials $(P_{\bar{x}})$ and mean power $(\bar{P}_{x_i})$ of electroencephalic activity*

1 kHz dB	$P_{\bar{x}}$ 0–511† (ms)	$P_{\bar{x}}$ 512–1,023‡ (ms)	$\bar{P}_{x_i}$ 0–511† (ms)	$\bar{P}_{x_i}$ 512–1,023‡ (ms)
10	101	22	70	50
30	398	18	77	55
40	600	17	88	58

*Arbitrary units, different for $P_{\bar{x}}$ and $\bar{P}_{x_i}$
†Post-stimulus periods.
‡Pre-stimulus periods
In interindividual comparisons the enhancement of $\bar{P}_{x_i}$ in the post-stimulus regions has been found down to 10 dB but statistically significant for dB values ⩾30 dB (5% level of significance, t-test of compounded samples only.)

[1] Sayers, B. McA., Beagley, H. A., and Henshall, W. R., *Nature*, 247, 481–483 (1974).
[2] Sayers, B. McA., and Beagley, H. A., *Nature*, 251, 608 (1974).
[3] Finkenzeller, P., *Kybernetik*, 6, 22–44 (1969)

SAYERS AND BEAGLEY REPLY—The power of an averaged sensory evoked EEG response (AER) can increase with suprathreshold stimulus level, as von Specht and Kevanishvili have recapitulated[1]. As pointed out by us[2], it often does so. The observation that even when this effect was negligible both an evident AER and a clear aggregation of Fourier spectral phases with repeated stimuli could still be seen, led us to investigate the relevance and use of phase spectra—our conclusions relate solely to that point.

But we disagree with von Specht and Kevanishvili[1] on three points. First, power changes in the AER cannot be used to deny a phase constraint hypothesis or, more specifically, to argue against the dominant importance of phase effects in respect of pattern that our results suggest.

Second, the evidence for the dominant importance of phase lies not in the extent of any AER power changes but in the finding that imposing the appropriate phase spectrum (that is, that of a suprathreshold AER) on a length of unstimulated EEG, converts this into a pattern highly correlated with the true suprathreshold response. Repeating the experiment by imposing the amplitude spectrum has no such effect.

Third, we think it inadvisable to base the detection of a true response on an assessment of average power in the post-stimulus EEG, in spite of attempts[3] to use that method in support of other procedures. In our experience, spontaneous EEG (especially in the young child, where the auditory evoked response test is of practical importance) is often neither low level nor free from substantial unpredictable power fluctuations. These affect both the power in the averaged response and the average power in the individual lengths of post-stimulus EEG, and so interfere with recognition of any possible response, especially near threshold. In spite of the attractive simplicity of a power method, we therefore abandoned it in favour of a pattern-detection method.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

On the first two points, we wonder if von Specht and Kevanishvili have overlooked the effect of increased coherence (in the sense of pattern similarity, between different lengths of on-going EEG subjected to a phase constraint on major spectral components) that could be expected to follow from increased suprathreshold stimulus level. The amplitude of the resulting averaged post-stimulus EEG would increase substantially, as we can demonstrate using von Specht and Kevanishvili's own results (Fig. 1). Figure 1 shows the result of averaging 30 records of pseudo-post-stimulus EEGs derived from a length of unstimulated EEG subjected to a phase constraint altered according to seven values of a parameter θ. This sets the extent ($\pm\theta°$) of uniformly random phase scatter (added independently and individually for each harmonic and for each of the 30 records) around the mean phase spectrum imposed—in this case, that of the 40 dB-stimulated example shown by von Specht and Kevanishvili[1] (their Fig. 1*a*).

The amplitude spectrum of the spontaneous EEG was retained unaltered throughout. Maximum pattern coherence is achieved for $\theta = 0$, and minimum for $\theta = 180$. Increasing the synchronisation of phase between records at repeated trials ($\theta = 180, 150, \ldots, 0$) in accordance with increased stimulus level thus greatly affects pseudo-AER magnitude and power, and is sufficient to account for the power increase in AER tabulated ($P_{\bar{x}}$). If different lengths of spontaneous EEG are used for each individual pseudo-response record, typical fluctuations of on-going EEG power can intrude. Some trials showed almost no increase in pseudo-AER power for θ-values as small as 80, because of fluctuations in on-going EEG. Others showed larger changes.

This explains our third point of disagreement, matches well our observations, and illustrates why we think that the small change in average power ($\bar{P}_{x_i}$) with stimulus level tabulated by von Specht and Kevanishvili[1] does not significantly support their view. Nevertheless, we do regard the additivity of an evoked response as a useful model; our suggestion is simply that it is increasingly less useful as stimulus threshold is approached, even if only because of the sporadic fluctuations of on-going EEG. Attention is drawn to the importance of phase-constraint effects which, once recognised, suggest other and more objective ways than averaging to detect an evoked response. The phase effects created by a successful stimulus are a property of signal patterns, and are not restricted to the ERA situation.

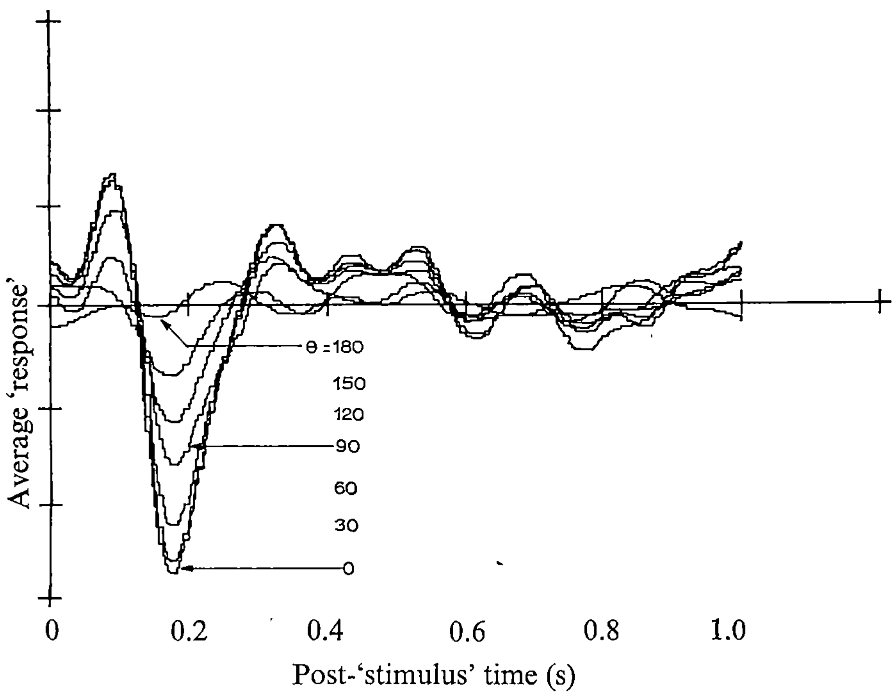

Fig. 1 Average of 30 records of pseudo-post-stimulus EEGs derived from a length of unstimulated EEGs subjected to a phase constraint altered according to seven values of the parameter θ (see text).

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[1] von Specht, H., and Kevanishvili, Z. Sh., *Nature*, 260, 461 (1976)
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reviews

Desert island crystallography

U. W. Arndt

Crystals, X Rays and Proteins. By Dennis Sherwood. Pp. xxii+702. (Longman: London, January 1976.) £12.50.

A (highly) intelligent school leaver about to take up an appointment as an X-ray crystallographer in a biochemical laboratory and wrecked on a desert island on his way, would find the present volume invaluable in equipping him for his new post by the time of his rescue. Dr Sherwood takes the reader in a methodical way through all the necessary steps to the solution of an unknown crystal structure. He starts with an explanation of the fundamentals of crystallography, of wave motion, of diffraction and Fourier transform theory and develops the necessary mathematical methods on the way. He then deals with the theory and practice of crystallographic structure determination—intensity measurement, extinction, Patterson methods, phase determination, refinement and direct methods are dealt with in successive chapters. The final section deals with biopolymers and diffraction by helical structures. In spite of the title, the chapter on protein crystallography as such is the least adequately covered. An appendix discusses a practical example of the solution of an organic structure.

The presentation is clear and fairly mathematical although it is a little doubtful whether it could be followed in its entirety by a reader who had not met any of the concepts before reading this volume. The author adheres throughout to a treatment in which he first states what he is going to say, then says it and finally discusses what he has said. The chapter summaries, in particular, are useful. Other good features are the annotated bibliography and the glossary and index. References are mostly to monographs and reviews, rather than to original papers The illustrations are plentiful and informative, although more modern X-ray photographs of biological materials might have been chosen. The text seems reasonably free of errors and misprints. It is a little strange to find within the same covers an illustration of wave interference in a ripple tank and a derivation of the Harker–Kaspar inequalities, but the author has fulfilled his stated intention of saying something of use for every class of reader in every section. Although the coverage of the theory of X-ray crystallography is fairly complete the reader will have to look elsewhere for a description of experimental methods.

This is a book to keep at home or in the office far from the departmental library, to read up on or verify X-ray crystallographic theory. It is of less value in a library since all the topics which the book covers can be found in readily available standard textbooks, although quite a number of separate ones may have to be consulted.

Do-it-yourself sedimentology

Brian Waugh

An Introduction to Sedimentology. By Richard C. Selley. Pp. xi+408. (Academic: London, New York and San Francisco, January 1976.) £5.90; $14.75.

HAVING recently returned to academic life after several years in the oil industry, Dr Selley has obviously seen the need for a textbook on sedimentology with a distinct emphasis on the more practical and economic aspects of the subject, particularly in its application to petroleum geology. As indicated in the preface, the book presents an overview of the more important fields of sedimentology in an attempt to satisfy the needs and interests of students and practising geologists. It should of course be remembered that sedimentology has evolved rapidly over the past few decades, and presenting an overview must therefore necessarily involve concentrating on concepts rather than detailed analysis Consequently, this book should be regarded strictly as a basic introduction to sedimentology in which subject matter is discussed at a very general level; but one which should also provide the reader with a reasonable background knowledge of the subject The practising petroleum geologist may well have appreciated a more advanced treatment.

The text is presented according to a logical format, commencing with the now familiar summary of the textural characteristics of sediments, including a valuable section on porosity and permeability. A short chapter on weathering is very disappointing, leaving the reader with little more than an elementary understanding of weathering and soil-forming processes. The sedimentary rocks themselves are covered in two chapters on allochthonous and autochthonous sediments, each major rock type being described with respect to classification, petrography and, where relevant, the relationship between diagenesis and porosity development. The sections on carbonates and evaporites are particularly useful summaries, although coverage of the other rock types suffers an inevitable brevity. Physical aspects of transportation and sedimentation are qualitatively reviewed, followed logically by a discussion of the morphology and origin of biogenic and inorganic sedimentary structures. A major section is devoted to a study of sedimentary facies and environments, on the basis of which the author erects a series of informative sedimentary models. Sedimentary basins, their origin, structure and evolution are discussed, including a few short case histories illustrating the sequence and variety of basin fill deposits. The final chapter deals specifically with applied sedimentology, highlighting the role of sedimentological knowledge in the exploration and exploitation of hydrocarbons, together with a tantalisingly brief discussion of sedimentary ores

The book is written in a very informal style, a feature which should appeal to the student reader at least, and the diagrams are on the whole clearly presented and informative. The major criticism of the book—that is, that too much has perhaps been attempted in this overview with the result that many topics are not discussed to any great depth—is perhaps counterbalanced, certainly for the more specialist reader, by the inclusion of most comprehensive reference lists.

Touching the right chord

Disease in a Minor Chord. By E. A. Steinhaus. Pp. xviii+488. (Ohio State University: Columbus, August 1975.) $20.

THE late Edward Steinhaus has come to be regarded as perhaps the most significant 'founding father' of invertebrate pathology. This book was intended, in his own words, to be "semi-historical and semi-biographical", but was never finished because Steinhaus died in 1969. His widow, Mrs Mabry Steinhaus and one of his colleagues at Berkeley, Dr Mauro Martignoni, however, wisely did not attempt to complete the manuscript but instead concentrated on preparation for publication. The result is a book, presented with care and sympathy, which makes for fascinating reading.

Readers not familiar with this field may be surprised to learn of the struggle that Steinhaus had to obtain recognition for the importance and scientific merit of studies on diseases of insects and other invertebrates. Insects alone account for about 75% of the known animal species in the world and are man's greatest competitor for food as well as transmitting some of his most troublesome diseases. Nevertheless, the study of insect diseases remained an esoteric subject known to few men and scarcely at all to science until the late 1940s. It was typical of the period that when Steinhaus required a class text on insect pathology, none was available, so he had to write one himself.

Chapter 1 deals with the early history of insect pathology reaching into Greek science and ending in Chapter 2 with developments in immediate post World War II period. These two chapters will become prescribed reading for any student wishing to study the history of insect pathology. In Chapter 3 the significant contribution made by Californian workers is recounted. The prose in this chapter is perhaps the best in the book and it is the one in which we begin to know Steinhaus as the man rather than a familiar name in the literature. We can share his frustrations and also identify with his ambitions. Anyone who has tried to develop novel and possibly unconventional ideas cannot fail to have understanding, sympathy, but above all admiration for the task that Steinhaus set himself in California. We can rejoice that these hopes and aspirations were largely achieved, although not without pain and anguish. It is true that many major developments in insect pathology have appeared since 1969 but nevertheless it must be acknowledged that the scene for these advancements was set largely by Steinhaus.

Chapter 4 presents an interesting, although inevitably incomplete, account of the European contribution to the knowledge of insect diseases, and in Chapter 5 we have Steinhaus' real and sometimes painful introduction to the intricacies of European scientific politics. Steinhaus the Editor was the creative force behind a scientific journal (*J. Insect Path.*, now *J. invert. Path.*) and an annual review (*A. Rev. Ent.*) which have for many years now been accredited with international standing and the background story to these developments is given in Chapter 6.

It says much for the style and presentation of this book that it can be read and enjoyed by professional and layman alike and as such can be recommended to all. **T. W. Tinsley**

Fungal development

An Introduction to the Biochemistry of Fungal Development. By J. E. Smith and D. R. Berry, pp. 326 (Academic: London and New York, 1975.) £9.50; $20.75.

ALTHOUGH few would deny that there are areas of molecular biology that require consolidation with further research, many molecular biologists (or are they microbial physiologists?) have turned to the comparatively virgin domain of microbial differentiation for a fresh challenge. Differentiation in micro-organisms is rather difficult to define, but fundamentally it entails the formation of morphologically different structures from vegetative organisms. Most microbiologists would go along with the great pioneer of research into biological differentiation, John Tyler Bonner, who believes that differentiation in organisms must be studied for the knowledge that it provides about those organisms. Nevertheless, many biologists who have turned to problems in microbial differentiation have done so because the system they have chosen to study provides a valuable model with which to study more complex metabolic differentiation processes that operate in higher animals and plants, the aim being to establish the basic mechanisms of control of gene expression as a cell switches from vegetative growth to formation of a differentiated structure. Not surprisingly, this upsurge of interest in microbial differentiation has generated texts and symposium proceedings, many of them excellent in their way although they are mostly multi-authored volumes.

The main attraction of the text written by Smith and Berry, which restricts itself to differentiation in fungi, in which so many of the attractive systems are to be found, is that it is of a piece and does not suffer from the 'bittiness' that comes so often with a collection of articles writen by different authors. The first of eight chapters deals with the conceptual basis of morphogenesis, and includes fundamental information on regulation of gene expression and enzyme action in cells. There follow two chapters on development in the slime moulds *Dictyostelium discoideum* and *Physarum polycephalum,* two very popular organisms for studying differentiation in multicellular systems. The fungal spore, the vegetative state, and asexual and sexual reproduction are the subjects of the chapters that follow, and the text concludes with a chapter entitled 'Differentiation, secondary metabolism and industrial mycology' describing production of compounds such as organic acids and antibiotics by moulds in what is essentially differentiation at the level of chemical synthesis as distinct from morphology.

The book has many attractive features. Each chapter ends with a brief summary, which will make it a valuable teaching text. Although at first sight there may seem to be too great an emphasis on fungal differentiation processes that have as yet been poorly researched, this is in many respects a richness for in this way the molecular biologist and biochemist can become acquainted with some of the more esoteric, but in differentiation terms very attractive, regions of mycology.

There are surprisingly few 1973 or 1974 references, which means that the book will quickly become dated. In general, Academic have done a fine job in producing the volume, although this was not helped by the authors electing to use line drawings of organisms rather than photomicrographs or electron micrographs, as well as some very old diagrams of life cycles. The text would have fulfilled its aim of providing an introduction to the biochemistry of fungal development if it had dealt in rather more detail with the molecular basis of regulation of gene expression and enzyme action. There is, for instance, no reference to specific degradation of enzymes or of enzyme inactivation by chemical modification such as adenylylation.

The attractive features of the text, however, far outweigh these reservations, and it should prove a popular introduction for the teacher and for the researcher who wishes to enter this most challenging and fast developing area of biological research.

Anthony H. Rose

Surface physics not clarified

Surface Physics of Phosphors and Semiconductors Edited by C. G. Scott and C. E. Reed. Pp. xiv+644. (Academic: London and New York, November 1975.) £18.80; $48.75.

THE appearance within a few months of two important books which attempt to summarise surface physics suggests that this previously murky field is now beginning to take on a clearer shape. One book (M. Prutton's *Surface Physics,* recently reviewed in *Nature* (**260,** 374; 1976) is a compact organised teaching instrument which deals only with clean surfaces; the other, named above, aims to give a much fuller picture, and caters largely for solid-state device physicists in pursuit of better electronic and opto-electronic devices. It is essentially a series of research reviews which, although they do not overlap greatly, do not interlock too well either.

The first 300 pages contain introductions to the theory and method of surface physics. Our understanding of surfaces has come mainly from studying specially cleaned surfaces; the structure of these has been determined by electron diffraction and spectroscopy whereas theory has been developed by extending the wave mechanics of bulk materials. This section leaves one wondering whether the experiment and theory will ever be brought together to form a proper picture of the electronic nature of a surface state, even that found at a clean vacuum–solid interface, leaving aside the much more difficult problem of the 'real' interface which is formed beneath a native or intentionally-grown oxide film. It cannot be said that this book really grasps that nettle. All the important facts are there but the authors perhaps do not work hard enough at building them into a coherent whole. To quote an example from the editors' own 100-page review of the compound semiconductors: we are told about the striking increases in surface-state concentration in gallium arsenide which are produced by washing the surface with copper ions. Why should this happen? Although the previous 80 pages has described extensive investigations of the surface of compound semiconductors, none of the 'feel' which that might have provided is used to clothe the new, intriguing experimental fact with a few shreds of explanation. One chapter, however, does take the reader gently by the hand; this is Zaininger's highly original survey of some principles of device operation which may be particularly vulnerable to the presence of unwanted surface states.

Although the term 'phosphor' appears in the title, the physics of luminescence is hardly considered at all. The greatest phosphor, zinc sulphide, in fact gets one of the shortest treatments in the book. Few gaps, however, are left in the solid-state microelectronics field (possibly Schottky barriers and electron loss spectroscopy get less than their due) and workers in this field will gain from seeing this set of reviews in juxtaposition. It will also stimulate the thought processes of any physicist working on surface effects and may even spur one of the next generation to write the book which at last pulls together the theory and practice of the physics of semiconductor surfaces.

Andrew Holmes-Siedle

New mind, new body

New Mind, New Body: Bio-Feedback: New Directions for the Mind. By Barbara B. Brown. Pp xiii+464. (Hodder and Stoughton: London, 1975.) £5.25.

THIS book is a well written, informative, popular introduction to biofeedback. Biofeedback is a method for training a person to increase his or her control over their own physiology. Barbara Brown effectively conveys the excitement and promise of this new field. *New Mind, New Body* is packed with information about biofeedback and about the way our mental and emotional selves interact with our bodily selves. To make her personal point (and this is one person's view of biofeedback) Dr Brown selects the evidence, but it's a good, thought-provoking selection. As a popular book, its style may be a little breezy and condescending for the specialist. The references are quite thorough and will be useful to the student and professional alike.

My personal fun was reading Dr Brown's rather caustic comments on the sterility of behavioristic psychology and its total inability to deal with the links between mind and body. She levels other criticism at mechanistic physiologists who have written of their speciality as if they had it in a jar completely isolated from the rest of the whole person. She points out what happened when behavioristic psychology mistook itself for the whole of liberal arts.

Dr Brown makes sure that her role in the "founding" of the Biofeedback Research Society is clearly documented in the early chapters. Researchers in the field knew it already but the public may want to know too. Succeeding chapters are each organised around a particular physiological system. Within each chapter various biofeedback studies are discussed. This makes a readable sequence but it is not, I think, an organisation which identifies the major themes of biofeedback. For instance, mind–body and mind–brain mechanisms for learning, special effects of feedback, parameters of practice, parameters of the external feedback path, biofeedback as a system, are not discussed as salient topics.

The coverage of the bioelectric response of skin, musculature, heart, blood pressure and brain (recorded as a scalp EEG) is comprehensive. Yet, laced through the factual reporting are interpretations which range from expressions of indignation and sarcastic commentary, scientific judgments, to personal and rather woolly kinds of theories. Dr Brown really believes in mind as an object which can be studied by public science. She insists that biofeedback offers a method and that the results of biofeedback research gives a new basis for accepting the mind as a demonstrated phenomena. Her arguments are spirited, and her intentions are clear—she is not trying to pull her wool over the reader's eyes. But it is also a fact that many biofeedback researchers would make very different interpretations of the same data. For example, Dr Brown clearly believes that EEG phenomena can reveal a lot about the mind and that unique mind states are linked to unique EEGs. Yet, most users of EEG would find it difficult to defend the more conservative statement that unique brain states can be revealed in EEGs. The consensus is that the EEG doesn't provide a clue to subjective, mental life, and is a gross representation of the electrical activity of the brain.

The importance of biofeedback for training one's own physiological processes is undeniable and Dr Brown was one of the first to recognise the whole implication of this new technique. She is absolutely correct when she projects what can happen when and if humans learn how to control their own physiology by means of biofeedback.

The importance of biofeedback lies in its efficacy as a training method. Whether scientists know or agree on "how the person does it" is not really relevant to its practical value in laboratory and clinic. In fact the benefits of biofeedback are independent of the mind–brain, mind–body issue.

All-in-all it's a book worth reading. It's a personal journal produced by a lively person, with an informed mind and fearless spirit. As a popular exposition it's excellent and far ahead of its nearest rivals.

T. B. Mulholland

Introducing mouse teratomas

Teratomas and Differentiation. (A Symposium held at the Roche Institute of Molecular Biology, Nutley, New Jersey, May 19–21, 1975.) Edited by M. I. Sherman and D. Solter. Pp. xv+324. Academic: New York and London, November 1975.) $16.50; £8.60.

ALTHOUGH mouse teratomas have been available as an experimental system for more than a decade, it is only over the past few years that the application of immunological, cell culture and embryo micromanipulation techniques has begun to realise their enormous potential for studying regulatory mechanisms in mammalian development. It was in this climate of optimism (not without an element of elite evangelism) that a meeting was held at the Roche Institute of Molecular Biology in May, 1975 to consolidate and assess recent gains.

This book consists of the papers presented at the meeting, and is by far the best introduction to the subject at present available. It is a pity that the book does not also include discussion following the papers, along the lines of the CIBA symposia, so that the reader can enjoy the frisson of dissention and the benefits of a wider range of opinion. This would have been particularly useful following the papers by Barry Pierce and Beatrice Mintz in which, *inter alia,* it is argued that mouse teratocarcinomas are an important model for the study of cancer since they provide a case in which malignant stem cells give rise to benign progeny by differentiation. It is implied that many other cancers arise by a disruption in normal cellular interactions which reduce the probability that the progeny of stem cells will differentiate, and that this non-genetic malignant change should be reversible if only the correct environmental signals could be imposed. 'Malignant', however, is never clearly defined and the relevance of mouse teratocarcinomas, which seem rarely to metastasise or invade, to the corresponding human tumour, to non-malignant hyperplasia or to chemical carcinogenesis, is not discussed.

Murine terato(carcino)mas arise spontaneously either in the gonads or from early embryos implanted into extrauterine sites. The comparative development of these tumours is clearly described by Leroy Stevens, who argues that the pluripotent stem cells are equivalent to the cells of the inner cell mass of the normal blastocyst Evidence in favour of this view comes from the pioneering work of the laboratories of Dorothea Bennett and Francois Jacob, which has shown that teratoma cells, sperm, morulae and blastocysts have in common a surface antigen which is probably coded for by one of the genes of the T locus. This work is the first step towards understanding the way in which early embryo cells may recognise each other by their surface properties.

Other exciting contributions are those of Gail Martin and Martin Evans on an *in vitro* method for obtaining relatively rapid and synchronous differentiation of homogeneous stem cells into endoderm, which in turn seems to trigger the next stages of embryogenesis, and the highlight of the meeting, which was the report by Beatrice Mintz that teratoma cells injected into normal embryos are able to participate in the formation of a normal, chimaeric mouse. The evidence for integration of teratoma cells into a wide variety of tissues, including germ cells of the testis, is impressive and the finding rich in biological implications. The book is worth reading for this paper alone.

Brigid Hogan

Iron transport and storage

Proteins of Iron Storage and Transport in Biochemistry and Medicine. (Proceedings of EMBO Workshop Conference on Proteins of Iron Storage and Transport.) Edited by R R. Crichton. Pp. x+454. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) Dfl.88; $36.75.

THIS book contains the proceedings of the Workshop Conference on Proteins of Iron Storage and Transport held by the European Molecular Biology Organisation in Louvain-la-Neuve in April, 1975. The main themes presented in the book are the molecular biology of transferrin and ferritin, iron transport and storage proteins, respectively, and the subcellular transfer of iron by the intestinal mucosal cell during iron absorption There is a total of 56 communications by contributors from Europe, the US and Australia.

In the section of the book devoted to transferrin all the outstanding problems related to its structure and function are brought to light and discussed. Two independent groups of workers presented almost the entire amino acid sequence of transferrin Several papers deal with the interaction of the metal and anion at the binding site, and demonstrate how iron and a ligand (with a carboxylic group) fit snugly into the receptor pocket on the protein surface. In addition, the distances between the iron, carbon and nitrogen (of the nitroxyl group) within the binding site have been measured using nuclear magnetic resonance and found to be about 1 nm. I find, however, that the most interesting investigations are not related to transferrin but to its specific receptor site on the surface of the immature red cell. The isolation and analysis of the transferrin receptor, a protein patch on the reticulocyte surface, initiated by Jack Fielding and Barbara Speyer, are bound to provide a solution to the biochemical basis of transferrin uptake and release by reticulocytes.

The contributions on the iron storage protein, ferritin, can be roughly divided according to their aim into the following groups. First, there are reports on the primary structure of this large molecule (molecular weight 450,000) composed of 24 subunits which form a shell enclosing a core of iron hyroxide and phosphate. In these reports the structure of subunits and their amino acid sequence are also described. Second, investigations on apoferritin subunit–subunit ineraction and assembly are presented These are followed by communications on identification of isoferritin in normal man and animals The significance of isoferritins in malignant diseases is then discussed Also, an atempt to determine the intracellular site of apoferritin synthesis and its turnover is described. Finally, but not least impressive, there is the work on iron uptake and release by ferritin carried out independently by Pauline Harrison *et al* and by two groups of investigators led by Robert Crichton and S Stefanini.

In the last section of the book several communications on the mechanism of iron handling by the intestinal mucosal cell during iron absorption are presented. The picture of subcellular iron pathways is not yet complete but intensive investigation under way in this field will no doubt bear fruitful results.

This book represents a faithful picture of the currents of research on iron metabolism carried out by investigators in many laboratories all over the world The book is published within a few months of the Conference and thus it has a rare quality of freshness. The editor's skill in balancing finely the material presented makes it an exciting book to read The reader with only a superficial interest in iron metabolism cannot easily appreciate the value of many hints so useful for bench work and may find many arguments too difficult to follow. For the iron enthusiast, however, this book is a 'must' and it certainly deserves space on a shelf of every reference library

B. Brozovic

Most studied single cell

Biochemistry and Pharmacology of Platelets. (Ciba Foundation Symposium 35, New Series.) Pp. viii+352. (Elsevier/Exerpta Medica/North-Holland: Amsterdam, Oxford and New York, 1975.) $23.95; Dfl. 59.50.

YET one more book on platelets! So prestigious a gathering as a Ciba Symposium, however, can be relied on to produce an authoritative book. What is the fascination that is fast making the platelet the most studied single cell? Partly, it is easily isolated yet more complex and interesting than the red cell: an understanding of it may contribute incalculably to the prevention of thrombosis. In addition, it is studied almost as a type cell The actin and mysin are similar to that found in muscle. Dr Cohen exclaims "the amazing thing is that fibroblasts respond to exactly the same stimuli as platelets do". Biological amines and monoamine oxidase drugs often have analogous effects on platelets and on the central nervous system. Another reason for the fascination is the close link of platelet biochemistry and pharmacology to platelet physiology. Indeed the contributors are as interested in pseudopod formation and sticky membranes as in the metabolic pathways involved: in fact large areas of platelet physiology are covered.

The basic membrane change involved in platelet 'stickiness' remains elusive but the membrane response to stimuli and the part played by the adenine nucleotides among many others, are presented in detail. The interrelationships between actin and myosin and the kinase of platelets and other cells are discussed. The pharmacology of platelet aggregation induction, its many inhibitors and the prostaglandins and aspirin effects are all well covered. The 'Platelet Release Reaction' has now become two: I from dense granules: II from α granules. Glycolysis, mineral elements, the part played by calcium and the gangliosides—it is all here including receptor action and bioamine storage.

Although not wishing to detract from the interest of the many detailed studies presented, one questions the relevance of some to intact platelets *in vivo*. They are so sensitive that demonstrable alterations occur, for example, with a poor venepuncture or after a fatty meal. Although the effect of prostaglandins on the biochemistry is extensively considered, I would have liked more space devoted to the prostaglandin E_1 precursors and platelet inhibition—that elusive therapeutic goal.

As usual the standard of production is excellent Professor Born and Ciba are to be congratulated, particularly on the editing of the discussion. But even so I don't find this nearly so rewarding as the papers themselves. This book is not easy going but is a recommended and masterly survey up to mid-1975 for physiologists and haematologists as well as biochemists and pharmacologists.

J. R. O'Brien

Learning theory

Elements of a Two-Process Theory of Learning. By J. A. Gray. Pp. x+423. (Academic: London, New York and San Francisco, 1975.) £6.60; $16.50.

IN the past twenty years the study of animal learning has suffered a sharp loss in prestige; in the 1950s it was seen as the most developed and 'scientific' topic within experimental psychology; and great, although perhaps unrealistic, hopes were held out that it would illuminate our understanding of human behaviour and personality. These hopes have not been realised and, with the development of the cognitive and information-processing approaches to human behaviour, animal psychology is often seen as an unattractive and somewhat stagnant backwater with few connections with the wider currents of psychological thought. Dr Gray has argued that the study of animal learning cannot be divorced from human psychology, and that it has much to tell us about human personality. In this book he still sees learning theory as providing a "scaffolding for a theory of the physiological basis of personality".

It is a pleasure to read a book which conveys such enthusiasm for the subject particularly when it is written with style and clarity; it is by no means an elementary book and makes considerable demands on the reader, but no-one who reads it could doubt that learning theory remains an interesting and challenging study, or that recent work on animal learning is providing valuable information about behaviour.

In his attempt to provide a unified account of a wide range of animal behaviour, however, there is a strong tendency to rely on a small number of 'crucial' experiments to support a particular line of argument; in some cases this may be justified, but in others there are a variety of other experiments, which pass unreferenced, that do not support the author's point of view. For example, the evidence on the effects of superimposing an appetitive conditioned stimulus on instrumental behaviour maintained by an appetitive reinforcer is confused and conflicting; there is at least as much reason to suppose that it suppresses instrumental responding as that it increases it as Dr Gray maintains. Rescorla is cited as supporting the idea of backward conditioning, where the experiments in question were concerned with simultaneous not backward conditioning. These are not isolated examples, but rather illustrate a weakness present throughout the book; the unwary reader may easily be misled in such cases. The author also has a tendency to attempt to resolve issues by definition or logic. Thus it is argued that the word drive is used to describe three rather different concepts which are logically and operationally distinct; it is then claimed that in the light of these distinctions the classical questions about the role of drive reduction in reinforcement largely disappear This logical coup, however, is achieved by making use of the concept of 'goal-directed drive', which is foreign to most forms of learning theory, and which, although undefined, seems to subsume all the goal-directed aspects of motivation. The subsequent logical argument merely spells out the consequences of the author's definitional decisions and takes our understanding of motivation little further.

The whole book is an attempt to provide a unified account of learning which the author describes as a two-process theory. It is curious therefore that the theory proposed relies very heavily on the classical conditioning component and, as the author admits, is very similar to Mowrer's 1960 theory, which Mowrer himself described as a one-factor theory. In Gray's, as in Mowrer's, model the instrumental component serves only to direct the animal towards or away from stimuli which possess positive reinforcing properties; and in spite of the earlier discussion of 'goal-directed drive', the way in which goal directedness enters into these relationships is not discussed. What we seem to have is a series of taxes, rather than an integrated goal-directed response. In short, the author provides a plausible account of how stimuli becomes attractive or aversive, but very little insight about instrumental behaviour.

I feel that these shortcomings make the book unsuitable for undergraduate reading; but this is a book which research workers in the field will find sometimes illuminating, sometimes irritating but always interesting

M. S. Halliday

obituary

Ben Dawes died at the age of 73 at his Burnley home on 3 March. He was formerly Professor of Parasitology at the University of London King's College, where he gave forty-two years of devoted service to the training of students and to research.

His first scientific appointment, after a hard-won start from being a weaver and then a tram cleaner, was to the Plymouth Marine Biological Station. Here he undertook research on plaice growth in relation to the availability of food, and his published papers on this continue to be standard references in fish feeding studies. In his early days at King's College he taught a wide spectrum of zoological subjects and in particular developed physiology and parasitology as final year undergraduate courses. His drive and enthusiasm were in no small measure responsible for establishing King's College as a centre of excellence in parasitology. His many students in this branch of study will remember his stimulating lectures and his well organised practicals, with an array of excellently prepared material from all parts of the world. Perhaps, too, his postgraduate students will have cause to recall his insistence on accurate compilation of data, on precision in presenting and writing up results and also his considerable disdain for the use of scientific jargon.

Professor Dawes was recognised internationally for his investigations into many aspects of the biology and physiology of the sheep liver fluke, and of particular significance were his studies into the penetration of host tissues, migratory routes, feeding, growth and drug effects on the immature stages in mammals and in snails. All this work was carried out with infinite patience, dedication and meticulous care: he drove himself very hard. His world-wide status as a helminthologist was enhanced with the publication of his book on *'The Trematoda'*, first published in 1946, reprinted in 1956, before being updated and re-issued in 1968. It still continues to stand the test of time as does his Ray Society Monograph on the trematodes of fishes. On assuming the editorship of the *'Advances in Parasitology'* series in 1963, Professor Dawes was insistent that it should reflect the progress in all areas of parasitology and the contents of the large number of volumes he edited demonstrate this resolve. He applied the same zeal, thoroughness and editorial criteria to each paper, irrespective of its specialisation, as he had to the work of his students, and always the advice and the criticism were intended to be helpful, a feature generally recognised by students and experienced workers alike. The 'cause' of parasitology was always near to his heart—a cause in which he lectured all over the world and in which he responded willingly to global requests for guidance and assistance. His demise leaves a gap in the ranks of world parasitologists.

announcements

Appointments

Dr Peter Ayscough has been awarded a personal professorship in Physical Chemistry at Leeds University.

Professor George F. Mitchell has been elected President of the Royal Irish Academy.

The Royal Society has elected the following as fellows: Professor Geoffrey Allen, Mr Sydney P. Smith, Professor Peter F. Baker, Dr Peter M. Biggs, Professor James Whyte Black, Professor Roger J. Blin-Stoyle, Professor Daniel J. Bradley, Professor John I. G. Cadogan, Professor William G. Chaloner, Professor H Charnock, Professor Patricia H. Clarke, Professor Geoffrey Eglinton, Professor Roger J. Elliott, Dr Lloyd T. Evans, Professor Albrecht Fröhlich, Professor Frank W. E Gibson, Dr Leonard G. Goodwin, Professor Edward G. Gray, Sir Ludwig Guttmann, Dr John M. Hammersley, Sir William MacGregor Henderson, Dr Robin Holliday, Dr John H. Horlock, Dr George B. Mackaness, Dr Dan P. McKenzie, Professor John F Nye, Dr Thomas G. Pickavance, Professor Hubert Rees, Professor Joseph M. Ritchie, Mr Stephen J. Robinson, Professor Howard H. Rosenbrock, Dr John D. Smith, Professor Stanley D. Smith, Professor Francis G. A. Stone, Dr Brian A. Thrush, Sir Frederick E. Warner, Mr William Watt, Dr Michael J. Whelan, Dr Elsie M. Widdowson, Professor Ronald K. S. Wood.

Awards

Dr E. F. Hartree of the ARC Unit of Reproductive Physiology and Biochemistry, Cambridge, has been awarded the CIBA Medal and Prize for 1975.

The Zoological Society of London has awarded **The Scientific Medal** for 1975 to **Dr W. D. Hamilton**, Imperial College of Science and Technology for his work on evolutionary biology and to **Dr S. H. P. Maddrell**, University of Cambridge, for his work on secretory mechanisms in insects.

Professor Arthur Kornberg of the Department of Biochemistry, Stanford University School of Medicine, has been chosen as the 1976 Jubilee Lecturer and recipient of the Jubilee Award of the Biochemical Society. Professor Kornberg has devoted much of his career to the study of enzymes involved in the replication of DNA. He was awarded the Nobel Prize in 1959.

The William Hopkins Prize of the Cambridge Philosophical Society has been awarded to **Dr S. W. Hawking** for his work on general relativity.

The Max Born Medal and Prize for 1976 has been awarded to **Hermann Haken,** Professor of Theoretical Physics of the University of Stuttgart for his work on the theory of lasers and also excited states in solids.

Academician **Ya B. Zeldovich** of the Space Research Institute Moscow has been awarded an Honorary D.Sc. by Sussex University. Amongst other topics, Dr Zeldovich has worked on Conserved Vector Currents, Relativistic Astrophysics, Shock Waves, High Temperature Hydrodynamics and Ultra-Cold Neutrons.

The Indian National Science Academy has announced the following awards for 1976: the Professor T. R. Seshadri 70th Birthday Meday to Professor R. C. Mehrotra of the University of Delhi, and the Silver Jubilee Commemoration Medal to Professor A. K. Sharma of the University of Calcutta.

International Meetings

May 12–13, **The Granulocyte: Function and Clinical Utilisation** (The Research Director, The American National Red Cross, Blood Research Laboratory, 9312 Old Georgetown Road, Bethesda, Maryland 20014).

May 19, **Scientific Research in Antarctica,** London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG)

May 27, **The Biology of Chemical Carcinogenesis,** London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

June 2–4, **Ozone Disinfection,** Chicago (Edward G. Fochtman, IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616).

June 4–5, **Isotope Effects on Enzyme-Catalysed Reactions,** Madison, Wisconsin (Dr W W. Cleland, Dept of Biochemistry, University of Wisconsin, Madison, Wi 53706).

June 6–9, **59th Canadian Chemical Conference,** London, Ontario (CIC, 151 Slater St, Ottawa, Ontario, Canada K1P 5H3)

June 6–13, **Zeolite '76,** Tucson, Arizona (F. A Mumpton, Chairman Zeolite '76, Dept of Earth Sciences, State University College, Brockport, New York 14420).

June 7–8, **Biological Monitoring of Water Quality,** Helsinki (EIFAC Secretariat, Food and Agriculture Organisation of the United Nations, Via delle Terme, 00100 Rome, Italy).

June 8–10, **Trace Substances in Environmental Health,** Columbia, Missouri (Dr D D Hemphill, Chairman, 411 Clark Hall, University of Missouri-Columbia, Missouri 65201).

June 8–19, **COSPAR Meeting,** Philadelphia (Dean P. Kastel, Space Science Board, NRC, 2101 Constitution Av., Washington, D.C 20418)

June 13–16, **Annual Meeting of the Society for Economic Botany,** Urbana, Illinois (Dr David S. Seigler, Dept. of Botany, 289 Morrill Hall, University of Illinois, Urbana, Illinois 61801).

June 21–23, **Mass Spectrometry in Drug Metabolism,** Milan (Dr A. Frigerio, Istituto di Richerche Farmacologiche 'Mario Negre', Via Eritrea 62, 20157 Milan, Italy).

June 26, **The Early Years of the Edinburgh Medical School,** Edinburgh (A. D. C. Simpson, Royal Scottish Museum, Chambers Street, Edinburgh EH1 1JF).

June 29–July 1, **Tropical Conference on the Weak Interactions,** Falmer Sussex (Conference Secretary, Dr E. A. Sanderson, M A.P.S., University of Sussex, Falmer, Brighton, Sussex BN1 9QH).

August 29–September 3, **Photobiology,** Rome (Deadline for abstracts: April 30) (Dr A. Castellani, Secretary-General, 7th International Congress on Photobiology, c/o Istituto Superiore di Sanita, Viale Regina Elena, 299, 00161-Rome, Italy).

September 7–10, **Meeting of the European Geophysical Society,** Amsterdam (Deadline for abstracts: June 15) (Mr C. R. Argent, EGS General Secretary, 6 Carlton House Terrace, London SW1Y 5AG, UK).

September 10–14, **Chapman Conference on Partial Melting in the Earth's Upper Mantle,** Southwestern Oregon (Deadline for abstracts: June 7) (AGU, 1909 K street, N.W., Washington, D.C. 20006).

September 27–29, **Pulmonary Macrophage and Epithelial Cells,** Richland, Washington (Deadline for abstracts: June 7) (Mrs. Judith A. Rising, Secretary to the 16th Annual Hanford Biology Symposium, Biology Dept., Battelle–Northwest, Richland, WA 99352).

October 21–23, **Annual meeting of the AGU Midwestern region–Eastern Section of the Seismological Society of America,** Ann Arbor, Michigan (Deadline for abstracts: July 27) (Meetings, AGU, Suite 1000, 1909 K street, N.W., Washington, D C. 20006).

October 30–November 5, **1st Panamerican Congress and 2nd International Cinematographic Review of Orthopaedics and Traumatology,** Mexico City (Deadline: July 31) (Paseo de la Reforma No. 445, 4th floor, Mexico 5, D.F.).

November 22–26, **Radiobiological Research Needed for the Improvement of Radiotherapy,** Vienna (Deadline for Papers: June 1) (Dr B. B Singh, Division of Life Sciences, International Atomic Energy Agency, Kärntner Ring 11, PO Box 590, A-1011 Vienna, Austria).

December 6–10, **Biological Applications of Stable Isotopes,** Leipzig (Deadline for papers: June 1) (Dr J. L. Servian, IAEA, P.O Box 590, Kärntner Ring 11, A-1011 Vienna, Austria).

Reports and Publications

Great Britain

Proceedings of the Royal Irish Academy. Vol. 75, Section A, No. 14. a Joint Browder Essential Spectrum. By M. Snow Pp 129–131. 24p. Vol 75, Section A, No. 15: Some Isoperimetric Bounds for the Torsional Rigidity of Anisotropic Elastic Cylinders. By J N. Flavin. Pp. 133–144. 40p. (Dublin: Royal Irish Academy, 1975.) [112

Molecular Aspects of Medicine, Vol 1, No. 1, 1976. Edited by H Baum and J. Gergely. Pp. 1–128 Annual subscription $40. Vol. 1 will consist of six issues to be published bi-monthly. (Oxford and Elmsford, NY.. Pergamon Press, 1975). [112

Agricultural Systems, Vol. 1, No. 1, January 1976. Edited by C. R. W Spedding Pp. 1–83. Subscriptions Four quarterly issues per volume, £15, $34 50. (London: Applied Science Publishers, Ltd., 1975.) [112

Institute of Food Science and Technology (UK). 1974/75 Report of Council, Council Committee Reports, Branch Committee Reports. Pp. 24. (Oxted, Surrey· Institute of Food Science and Technology, 1975.) [112

Chemistry and Industry Buyers' Guide 1976 Chemicals/Manufacturing Plant/Laboratory Equipment/Services/Scientific Book Publishers Pp. 86. (London. Society of Chemical Industry, 1975.) [212

The British Library. Scientific Research in British Universities and Colleges, 1974–75. Vol. 1. Physical Sciences Part 1 (Sections 1–36). Pp. xxii + 1–862 Part 2 (Name and Subject Indexes) Pp. xviii + 863 – 1146 £14.80 net (not sold separately) Vol. 2 Biological Sciences Pp xxii + 885 £11 25 net. Vol. 3· Social Sciences (Including Government Departments and Other Institutions) Pp. xxxii + 844 £10 65 net (London· HMSO, 1975.) [312

Pricing for Pollution· An Analysis of Market Pricing and Government Regulation in Environment Consumption and Policy. By Wilfred Beckerman. Pp. 72 (London The Institute of Economic Affairs, 1975.) £1. [312

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Advice and Responsibility. By Lord Zuckerman. (The Romanes Lecture for 1975.) Pp 40 (Oxford· Clarendon Press; London: Oxford University Press, 1975.) 95p net [412

Philosophical Transactions of the Royal Society of London A Mathematical and Physical Sciences Vol 280, No 1295: Asymptotic Solutions of the Orr-Sommerfeld Equation. By J M. De Villiers. Pp. 271–316. (London The Royal Society, 1975.) UK £1 95; Overseas £2 [412

Department of the Environment Community Land—Circular 1· General Introduction and Priorities. (London· Department of the Environment, 2 Marsham Street, SW1, 1975) 35p. [512

Science Research Council Solid-State Devices A strategy for SRC research support Pp. 17. (London. Science Research Council, 1975.) [512

Science and Survival. (SISCON· Science in a Social Context.) By Ernest Braun and Davil Collingridge.) Pp 54. (Leeds· Siscon Project, University of Leeds, 1975.) [512

University of Nottingham School of Agriculture Report 1974/1975 Pp 166 (Sutton Bonington, Loughborough, Leicestershire· University of Nottingham, School of Agriculture, 1975.) £1 20 [812

The Royal Society. Scientific Research in Schools Committee—Report to Council 1975. Pp 14 (London The Royal Society, 1975.) [812

Grasslambs An Intensive System of Lamb Production from Temporary Grassland By N E. Young and J E. Newton (Farmer's Booklet No. 1) Pp 28 (Hurley, Maidenhead The Grassland Research Institute, 1975.) [812

Memoirs of the Royal Astronomical Society Vol 80, Part 2 A Gravitational Three-Body Scattering Experiment. By M. J. Valtonen. Statistics of Three-Body Experiments—Probability of Escape and Capture. By M J Valtonen Pp. 61–91 (Oxford and London· Blackwell Scientific Publications, 1975 Published for The Royal Astronomical Society.) [812

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Swaziland, 1972/9713. Pp. 30p. Kenya, 1973/1974, Final Report. Pp. 61. 30p. Northern States, Nigeria, 1973/1974, Final Report. Pp. 61. 30p. Tanzania, 1973/1974, Final Report. Pp. 127. 30p ULV Spraying for Cotton Pest Control. (Report on a workshop held at Lowveld Experiment Station, Big Bend, Swaziland, 3 to 7 March 1975) Edited by N. Morton. Pp. 53 (London Cotton Research Corporation, 14 Grosvenor Place, SW1, 1975.) [1012

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Agricultural Research Council. Annual Report of the Institute for Research on Animal Diseases for 1975. Pp. 66 (Compton, Near Newbury, Berkshire Institute for Research on Animal Diseases, 1975.) [1512

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Person to person

NASA is continuing to encourage and support research on all aspects of lunar science and especially encourages well-qualified scientists not now in the lunar programmes to participate if they have new ideas, techniques, or research capabilities. An announcement giving details on where and how to propose may be obtained from Dr Edward A. Flinn, Deputy Director, Lunar and Planetary Programs, Code SL, National Aeronautics and Space Administration, Washington, DC 20546.

There will be no charge for this service Send items (not more than 60 words) to Martin Goldman at the London office The section will include exchanges of accommodation, personal announcements and scientific queries We reserve the right to decline material submitted No commercial transactions

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Australia· Commonwealth Scientific and Industrial Research Organization Division of Tribophysics Report, 1973/1975 Pp. 35 (Melbourne CSIRO, 1975) [3112

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Canada Department of Energy, Mines and Resources. Geological Survey of Canada. Memoir 380 Quaternary Geology and Geomorphology, Nicola-Vernon Area, British Columbia. By Robert J Fulton Pp xi + 50 $8 40 Paper 74–36 Franklin Bay and Malloch Hill Map-Areas, District of Mackenzie By C J. Yorath, H R Balkwill and R W Klassen Pp. vi + 35 $4 20 Paper 74–53 Surficial Geology of Tuktoyaktuk, District of Mackenzie By N. V Rampton and M Bouchard Pp v + 17 $4.20 Paper 74–54 Geology of the Precambrina Ramah Group and Basement Rocks in the Nachvak Fiord-Saglek Fiord Area, North Labrador By W. C Morgan Pp. vii + 42 $3.60. (Ottawa· Information Canada, 1975) [3112

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World Health Organization Technical Report Series, No 578 Radioimmunoassay of Hormones for Clinical Trials of Fertility Regulating Agents in Developing Countries—Report of a WHO Meeting of Experts. Pp. 24. Sw Fr. 5. Public Health Papers, No. 63: Schizophrenia—a Multinational Study. (A summary of the initial evaluation phase of the International Pilot Study of Schizophrenia.) Pp. 150. Sw Fr. 16. Monograph Series, No 55 Laboratory Techniques in Brucellosis Second edition. Pp. 163. Sw. Fr 35 (Geneva WHO; London. HMSO 1975.) [51

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Canada· Department of Energy, Mines and Resources Geological Survey of Canada Paper 74–40: Geology of the Lower Paleozoic Formations in the Subsurface of the Fort Simpson Area, District of Mackenzie, N.W T By N C. Meijer-Drees. Pp v+65 $4 20 Paper 75–31. Borehole Geophysics Applied to Metallic Mineral Prospecting—a Review By A. V. Dyck, P. J. Hood, J A Hunter, P G. Killeen, A. Overton, A M. Jessop and A. S Judge. Pp. 65 $3. Index to Publications, 1959–1974. Pp 138 $4.20. (Ottawa: Information Canada, 1975) [151

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nature

April 8, 1976

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Superstar technology monitored...or throttled?

SUPERSTAR technology is the name given by a working party of the Council for Science and Society to highly innovatory, large scale technical projects. A report just issued (*Superstar Technologies*, 66pp, £2.00) looks at "the social mechanisms by which critical expertise can be concentrated on [such technologies], so that grave defects and dangers, technical, environmental and social can be anticipated and minimised". The problem is said to be that there are inadequate mechanisms, particularly at the conception stage of superstar technologies, to monitor possibly disastrous consequences; this is partly because the technology may practically monopolise the employment and commitment of relevant experts, thus weakening the forces of criticism or even commercial competition, and partly because these relevant experts will, being only human, suffer from occasional frailties of intellect, conscience and vision. How, asks the council, can we spot any weaknesses before the project is irresistible?

The answer, with a certain amount of hand-wringing about yet another public institution, is a Technology Implications Commission (TIC) which would "foster the discovery, assessment and diffusion of reliable information about proposed advanced technical projects, so as to provide conditions for effective monitoring, with appropriate public participation at all stages of development". The TIC would neither replace nor interfere with present monitoring facilities further down the line such as select committees or inspectorates. Information would come to it by way of scrutiny of the technical press, patents and so on, "on the lookout for items whose significance might easily be ignored". It would also use independent consultants, and would be a place to which a 'whistle-blower' could turn. The TIC would, if there seemed to be a *prima facie* case for a possible danger, pursue its own independent research and could follow this up, if necessary, either by publicising its findings or holding a conference to generate public debate. The TIC would not have legal power to force the adoption of any policy or the abandonment of any project.

It would be pleasing to be able to give such a modest and mild proposal a wholehearted welcome, firm in the knowledge that it had been hammered out by a representative group after extensive international inquiries. Careful reading of the report suggests otherwise and casts some doubts that the council put together the right sort of mixture. The working party comprised a lawyer, an architect, a professor of physics, a physics research student, a professor of materials science and two science writers. A curious group, markedly, maybe fatally, deficient in practising technologists who might be presumed to know superstar technology from the inside.

It is sad also to relate that the concept of a superstar technology never really comes into focus. Various apparent candidates put in anecdotal appearances—Flixborough chemical works, the DC-10, Ronan Point (a high-rise block of flats that was severely damaged by a gas explosion), high alumina cement, the Comet, natural gas, supertankers, motor cars, large electrical generating sets, organic chemicals, Browns Ferry, Concorde, boxgirder bridges, thalidomide, Windscale and even groundnuts. All of these are, of course, coupled with disasters, and a mood of disaster informs much of the report. We are told that "the personal safety of thousands . . . and the prosperity of millions may depend upon the engineering success and environmental acceptability of a single enormous project". But the real superstar technologies, on this reckoning, are probably things of the past—railways, electricity and gas distribution, tunnels, sewers, steamships, telephones and transatlantic flight.

The working party claim that changes in the social and financial organisation of industry have weakened some of the forces controlling innovation; Brunel or Edison, for instance, carried heavy personal responsibility for safety and success, which was a continual check on their projects. This is a doubtful argument; Brunel and indeed all Victorian engineers lived with disaster and failure on a scale unknown these days. Society has since then gradually deemed human life less expendable and commercial failure more ignominious. But there has to be a limit to the amount of restraint (even if only moral) on a technologist's freedom of action or else he will give up practising or emigrate. The TIC might come dangerously near to snuffing out what enthusiasm a technologist has.

It is asserted that the control of advanced projects on behalf of society "must depend on the same principle as does science"; this means peer-review and mutual criticism. However well this has served science, it is doubtful whether scientists should go round trying to impose their way of reaching decisions on another community, where decision making is not just a matter of establishing the facts but also of assessing political, social and economic factors. And even 'the facts' are of a different order in technology—the report acknowledges as much when it speaks of the almost impossible task of judging the real weight of innumerable, often contradictory, warnings.

The real danger in all this is, first, that a monitoring agency be generated with which technologists cannot live and, second, that the institutionalising of monitoring may actually create a false public impression that everyone else can relax a little.

There are lots of good intentions behind the council's initiative, and few would quarrel with a call for more open decision making. But the TIC, for all its worthy beavering, might just prove the last straw that broke the not-entirely-wicked camel's back. □

Time for a reappraisal at the Select Committee

THE House of Commons Select Committee on Science and Technology is always an easy target for criticism. It has little money, limited time, few administrative resources. It possesses no legislative power, and shows a frustrating inability to influence intractable government departments.

This bleak picture is made worse by the committee's history of ill-prepared reports and badly formulated proposals, themselves often the product of seemingly sloppy if not uncaring hit-and-miss investigations unbecoming of serious parliamentary procedure. (Remember how the committee championed the ill-fated proposal to appoint a Minister of Research and Development?)

To cap it all, the committee last week published its report on a visit recently made to the Gulf states of Abu Dhabi and Kuwait to investigate "matters of oil policy and related energy questions". The somewhat inappropriate main recommendation was that more effort should go into the export of Range Rover automobiles to Arab countries. But more disturbing was its remark (an attempt at vindication?) concerning the British-built Steam Generating Heavy Water Reactor (SGHWR): "We were puzzled by reports that the . . . SGHWR cannot be submitted to overseas buyers until a commercial size reactor has been successfully constructed at home."

The committee should be familiar with the problems that have arisen with the Advance Gas Cooled Reactor because of premature scaling-up, and should know that present policy consequently demands stringent, highly detailed design specifications, still not achieved with the SGHWR. The chairman of the committee attempted to justify its thinking with the observation that Britain should not worry if other states are prepared to accept safety standards less stringent that its own.

The committee's approach suggests that it has become as disillusioned with its own role as everybody else has; an attitude of cynical self-disdain does disservice to British science and technology. The need for well informed, disinterested guidance is perhaps greater now than ever before.

Nobody can be expected to take this committee more seriously than it will take itself. It is time for a reappraisal, a move towards the professionalism and rigour customarily associated with the (admittedly better off) Congressional committees on the other side of the Atlantic. It does not require bottomless coffers and endless time to stop and consider the difference between disinterested and uninterested. □

Seeking sense about scientists in government

The debate over Civil Service scientists in the UK continues. Harold Turner of the National Physical Laboratory gives his view and takes up some of the issues raised in Nature's *columns by Cyril Cooper last November and David Budworth in February.*

CIVIL SERVICE baiting has become a popular national pastime. The points raised in relation to the science aspects of the sport fall into two main categories: first, those which question the role of the Scientific Civil Service (SCS) within British science and the British economy generally; and, second, those that are concerned with the organisation, structure and competence of the SCS itself. Any evaluation of the criticisms of the SCS must first look at the broader, overwhelmingly more important issues in the former category.

The growth of the SCS arose, not from a doctrinaire belief in Government science, but from the need to provide national scientific services, such as standards and geological surveys, and, in particular, from a desire to compensate for the failure of industry to undertake and apply its own research and development. The beginnings of the decline of investment in British manufacturing industry, and its replacement by the more immediately profitable alternative of investment abroad, had been perceived by some as early as the 1860s, and the First World War finally brought home to Government that much of British industry was so backward as to bring national survival into question.

The steps thus set in motion—to encourage research in industry, to set up Research Associations and Government research establishments—have led to the pattern we see today. In a very few areas, notably chemicals, industry itself now mounts a research and development effort comparable with that of our major competitors. In a few others, contributing only a small part of our industrial output, there is (for reasons linked with present or past defence policy) an overwhelming concentration of government support. For the rest, representing some 85% of our industrial output, there is an effort which is inadequate by any standards. This maldistribution of effort has persisted for a considerable time, through many successive governments. The failure of our large research and development expenditure (about 2.3% of GNP) to produce any comparable improvement in economic performance, can be seen to be due to an excessive concentration on high technology, to a general failure on the part of industry to undertake and apply its own research and development, and to a failure of both Government and industry to secure the application in industry of research and development carried out in the public sector and the universities.

In the late 1940s many people, including myself, believed that the technological and competitive weakness of much of British industry could be overcome through a research effort in which Government research establishments played an important part: that the successes of wartime research could be repeated in peacetime conditions. We believed that if we provided research and perhaps some of the development, industry would provide the production and marketing expertise, and investment and economic growth would follow. We thought that our contacts with industry and the involvement of industrialists in the work would ultimately ensure its relevance.

Now, sadly, nearly 30 years later, after passing through phases in which science has been alternately oversold ("the white-hot technological revolution") and undersold (as in the present phase of disillusion), we are wiser and sadder. We know now that the problems of industry are not primarily technological, but economic and managerial, and that we as scientists can have little influence upon them. Many of us now feel that when management and direction in industry is effectual, science will be undertaken or commissioned and applied, but that when management is ineffectual it will not.

The years since 1965 have seen an agonising reappraisal of the role of Government science and particularly of industrial research. There is now probably a reasonable consensus on the rightness of a Government presence in some research and development areas. Thus the provision of certain services, such as standards and specification, would scarcely be questioned. Similarly with work *for* Government, to provide advice as a basis for statutory or regulatory action (including taxation) and in connection with the procurement function of Government and public authorities generally. Most work for agriculture is welcomed.

But on the crucial question of innovation and growth in British industry there is still violent disagreement on what is the role of Government money and Government science. It is a matter for regret that the Confederation of British Industry (CBI), which could contribute so much, has so far treated these problems at such a superficial level. The SCS seems to have become the national scientific scapegoat. It is dissected and examined as if the solution of all our difficulties is to be found in its entrails. At the back of most official enquiries into the SCS is the feeling that something must be wrong with the organisation and the people if so much expenditure in the end produces so little economic growth. This is not only unjust to the able and dedicated people who constitute the great majority of the staff, but dangerous in that it poses a false and facile solution to a complex national problem.

Take the argument that the SCS is overstaffed and deprives industry and education of much needed scientific manpower. The CBI has campaigned for cuts in the SCS. But it is difficult at this time to take seriously the suggestion that the recruitment of scientists into industry and the schools is limited only or even mainly by the insatiable designs of the SCS. SCS recruitment has been at a low ebb for years, but even so the uncertainty about the future of the service has resulted in a considerable shortfall on advertised vacancies. If industry is not recruiting it is either by intention or because many young people find industry unattractive, making the issue one on which the CBI might take useful action, for the fault is unlikely to be wholly on the students' side. The problem of science teachers is quite separate: the financial rewards compared with either the SCS or industry must be an important factor.

The suggestion that the SCS is overstaffed is less a valid statement about the staff required for current programmes than an argument for the reduction of the scope of the Service. Sixty per cent of government scientists are employed on defence. Their work is closely related to the Government's procurement function in this area. Staff cuts have already been made and much larger ones seem likely in the next few years. The remaining civil areas have also been cut. Some areas of 'interventionist' work have already been dropped. Attempts to pin down the CBI to say exactly where the line should be drawn between government and industrial research and development have often brought a disappointingly vague response.

Then there is the argument that the SCS as a career service brings seclusion, stagnation, immobility and excessive security. The SCS is a career service because it was created within the Civil Service. The suitability of this form of organisation has often been considered, but the CBI (in evidence to the Fulton Committee) did not advocate the abolition of the career SCS, merely the greater use of short term appointments within it. A proposal to recruit all CS scientists to short term contract posts, with the retention of about 20% after 5 years, came from one individual, Sir Frank Turnbull, a former career administrator at the Department of Education and Science. This proposal was conscientiously considered but rejected by the Fulton Committee. The dangers of stagnation in a career service such as the SCS, though often talked about, have never been investigated "on the ground". There is little to suggest that unproductive scientific civil servants are more prevalent or more of a problem than their counterparts in other walks of life. Where they do exist they are all too often the end product of a chain of management blunders.

This is not to argue that the age structure of the SCS is ideal. However, the crucial factor is not that the numbers have been static for twenty-five years, but that the rapid recruitment in the 1940s and 1950s, falling away in the 1960s and 1970s, has led to an age distribution in which the proportion of older staff is higher than one would choose if there were no constraints. Too many able and effective senior men are inadequately supported. Retirement and movement are already tending to restore the balance, but in the long run the only satisfactory solution will be consciously to relate recruitment to the long term need for staff at the various levels. Once a long-term view of Government science is evolved this will not be difficult. At the moment, actual interchangeability with industry is still limited and movement out of the SCS is still relatively infrequent. But it may be questioned whether movement does have the unique importance so far attached to it when in some countries, such as Italy and Germany, more successful than we in applying science, it is even less prevalent than here. Movement of scientists in industry to non-research activities is common, but the analogous movement within the Civil Service remains at a very low level despite the recommendations of the Fulton Committee.

Finally, the argument that the SCS is overpaid and provides careers far more favourable than industry. It has been claimed that the typical graduate entrant to the SCS reaches Principal Scientific Officer, and by his late 30s at that. In fact, only around 50% achieve this grade before retirement (a sore point with many SCS graduates), and not all even reach the next lower grade of Senior Scientific Officer. Scientific civil servants are not life-long bench workers; in the defence area particularly, large numbers are occupied in the management of projects funded by government but carried out elsewhere. The role of these members of the Science Group and the nature of their responsibilities has no exact counterpart in industry. It is of course legitimate to compare the SCS with industry, but the chief difficulty in doing so is that while SCS pay and career details are fully accessible to the public, those of industry are not. Information is supplied by industry to the Civil Service Pay Research Unit, but only in the strictest confidence. Comparisons are further complicated by the major differences in the structure of careers between the SCS and scientific employment in industry. In addition there is evidence of a wide scatter of industrial rates. Recent surveys of the remuneration of Chartered members of professional institutions show great differences *within* industry, between different sections of industry, between different locations of employment and between different functions, notably between the scientific worker and the manager of scientific work. An informed discussion of the pay and careers of scientists in all sectors of employment would be welcome, but it ill-becomes the spokesmen of industry to attack the SCS while withholding information on which this discussion alone can be based. □

Rediscovering Islamic Science

An exhibition of Islamic Science opens this week in London—one of many displays, concerts and meetings organised by the Festival of Islam to give a perspective on the Islamic world. **David Davies** reports

It is very easy to view the historical development of science in terms of the contributions that separate nations or civilisations have made to what we now regard as real, successful, all-encompassing Western science. We get decimals from one place, the lodestone from another, paper from a third and so on. But knowing the history of science only in terms of what we got out of where is like knowing world geography only in terms of what different countries export—a common feature, it used to seem, of school textbooks. In order to understand Islamic science properly, one has to try and identify with Islamic needs and beliefs, and if one is thereby led into alchemy and astrology as well as water-engineering and mathematics, this was no less science in the Islamic world. "We have tried," say Francis Maddison and Anthony Turner in the introduction to their catalogue of the exhibition, "in all modesty, and albeit imperfectly as non-Muslims, to picture Islamic science and technology from within, that is, as part of the intellectual and everyday life of a widely heterogeneous community founded on a common faith."

Wide indeed; the Islam world stretches from Spain to Indonesia, from Samarkand to central Africa. And in time the exhibition, at London's Science Museum, covers nearly a millennium from the first infusion of Hellenic knowledge in the eighth century to the fading away of a specifically Islamic science in the sixteenth and seventeenth centuries. The word science in the exhibition does not, of course, have its present professional connotations of making hypotheses, verifying or falsifying them. It is much more the science-museum/popular view of science as a body of knowledge about things. As far as we know, the doing of experiments in a systematic way was as unknown in the Islam world of the middle ages as it was in the western world of the same period.

Two things cemented the Islam world: the dominance of two main languages, Arabic and Persian, and the importance of religion in everyday life. From religious needs sprang much of the science that will be on show. The great eleventh century philosopher al-Biruni commented rather tartly that people were paying more attention to their food, which was a twice-daily concern, than to their prayers, which occurred five times a day. It was in the correct ordering of these prayers that science had a major part to play, both in the accurate measurement of time and in determination of the direction of Mecca so that the prayer mat should be correctly aligned. Thus flourished clocks, sandglasses, astrolabes, astronomical tables, spherical trigonometry and so on. But at the same time there is astrology which was, at a later date in Western science, practised by some but which scandalised others.

The Islamic world stretched east to Indonesia but there is little evidence remaining beyond India of scientific achievements. But Muslims were great travellers, and Egypt and Syria in particular managed at the crossroads to hear about everything.

The exhibition has posed particular problems of what to display. To the scholar the most important sources are the manuscripts, of which there are large numbers. But in planning the exhibition Maddison and Turner have had to keep manuscripts to a minimum simply because they would be generally incomprehensible to a London public. Astrolabes were relatively easy to accumulate for most periods, but the difficulty has been in putting together a representative sample of other objects to exemplify the extent of knowledge. It has turned out that the richest sources of exhibits have been Paris, London and Oxford museums, rather than the Islamic countries themselves. A major disappointment, however, has been that Turkish law forbids even the temporary export of materials from particularly fine collections.

Prosperity in the Islamic world was largely determined by the availability of water, and so the exhibition has had to take note of water technology. But some of the most fascinating examples only existed in manuscript form—notably in al-Jazari's thirteenth century *Book of Knowledge of Ingenious Mechanical Devices*. No one, as far as is known, has constructed any of these devices for seven hundred years, but the recent publication of Donald Hill's splendid translation (Reidel, 1973) has awakened a wide interest in them. A huge water-clock built specially for the exhibition according to al-Jazari's specifications will undoubtedly be the central attraction for most people. Equally noteworthy, however, will be the half-size model of a water pump driven by a water wheel, also described by al-Jazari. The original was the first recorded pump to be powered other than by human or animal-power.

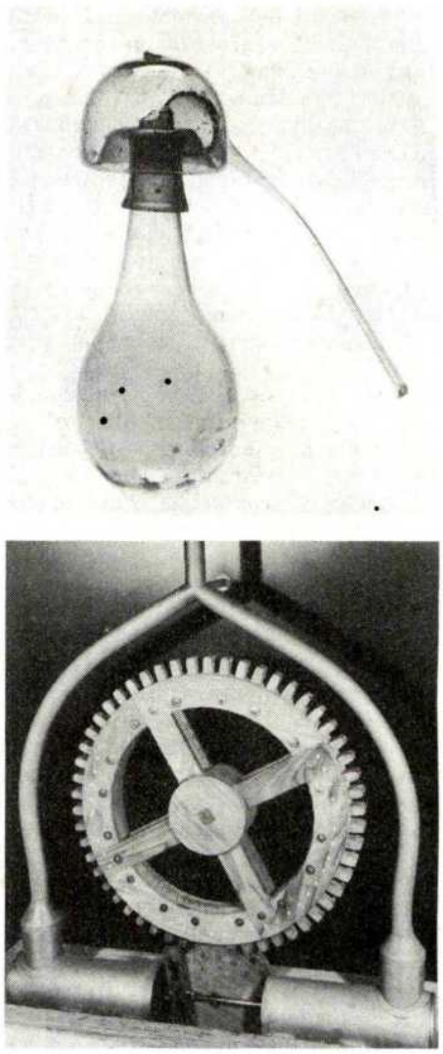


Photo: Mike Freeman

Photo: Science Museum

Modern alchemist's still (above) and (below) the model of a water pump driven by a water wheel

It is difficult to date the decline of a specifically Islamic science. The growth of the Ottoman Empire in Turkey helped to extend the period, and, both through Turkey and Spain, aspects of Islamic science were siphoned into Western civilisation.

There seems little doubt that the Islamic exhibits being brought together in London, not only of science, but of art, architecture, musical instruments and books, represent a unique occasion. And few believe there will ever in the foreseeable future be another occasion on which so much of the Islamic world can be seen in one city. □

The exhibition at the Science Museum is open daily until August 29. Admission 30p; children, students and pensioners 15p.

From embargo to control

A committee which has been striving for the past year to draft guidelines to control the use of a revolutionary, but currently embargoed, genetic engineering technique, last week paved the way for many uses of the technique to be resumed in the United States. Colin Norman reports from Washington

FOR nearly two years, many experiments involving the use of a newly-discovered method of manipulating genes in living organisms have been under an embargo because of potential hazards associated with the research. But scientists in the United States who have been itching to use the technique may not have much longer to wait, for it now seems almost certain that the embargo will be lifted in the next few weeks and be replaced with controls designed to minimise the hazards.

The latest step in the tortuous process of developing those controls occurred last week when a committee of the National Institutes of Health (NIH) met to consider criticisms and proposed revisions of a set of detailed guidelines it drafted last December.

The committee stood solidly behind its work, resisting most suggestions that its draft guidelines should be made more restrictive, and it also gave its approval for one particular biological safety system to be used for some of the more hazardous genetic manipulation experiments.

The matter is now firmly in the hands of NIH Director Donald S. Fredrickson, who last week promised to issue a final set of guidelines within a month. Though Fredrickson will probably amend the draft guidelines, a statement he sent to the committee last week indicates that he has accepted the principles embodied in the draft and that any revisions he may make will be relatively minor.

The draft US guidelines seek to contain bacteria or viruses bearing transplanted genes by two methods. First, they spell out four levels of physical containment, ranging from the use of standard microbiological practice to the use of specially designed laboratories (such as biological warfare establishments) capable of handling the most virulent pathogens, and second, they specify that experiments with high potential risk should use viruses or bacteria genetically crippled so as to be virtually incapable of surviving outside the laboratory. The central, and most controversial, portion of the guidelines attempts to assign specific experiments to specific safety levels.

After the committee finally hammered out those guidelines at a meeting last December, Fredrickson sent them out to several scientists for review, and he also called a public meeting to solicit the views of outside groups. The result is a stack of comments more than a foot thick, on the basis of which Fredrickson last week suggested some specific revisions in the draft guidelines and asked the committee for its reactions.

Many of Fredrickson's suggestions simply removed inconsistencies in the draft, and were readily accepted by the committee. But in several instances where Fredrickson proposed changes which would have made the guidelines more restrictive, the committee demurred, arguing that its draft guidelines would provide ample protection against the potential hazards. Specifically, Fredrickson suggested that experiments involving the insertion into bacteria of uncharacterised genes from cold-blooded vertebrates should be placed in the same category as experiments with genes from higher animals (next to the highest level of physical containment, coupled with the use of crippled micro-organisms); he asked the committee to consider the suggestion that genes from other cold-blooded animals and lower eukaryotes should only be transplanted into crippled bacteria; and he also asked the committee for its views on placing all genetic manipulation experiments with the monkey virus SV40 in the highest level of physical containment. Though the committee turned down all those suggestions, it remains to be seen whether Fredrickson will accept its views.

Whatever decisions Fredrickson makes on the fine details of the guidelines, it is clear that many genetic manipulation experiments will require the use of crippled viruses or bacteria before they are allowed to go ahead. In that regard, the committee last week took one important decision and came close to making a bad political blunder on a second.

At its meeting last December, the committee agreed that it should certify whether or not specific strains of viruses or bacteria have been sufficiently crippled to provide the degree of containment spelled out in the guidelines (it took on that assignment largely because no other competent national body is available). At last week's meeting, it was asked to certify two such crippled strains. If it approved them, the effect would be to give the green light to many experiments as soon as guidelines are published; if it turned them down, the embargo on experiments requiring crippled strains would effectively be maintained because no such strains would be available.

The first strain considered by the committee was a bacteriophage lambda, rendered incapable of surviving at body temperature and carrying three other genetic mutations which limit its infectivity and prevent it from surviving outside an artificial laboratory environment. Developed by Dr Philip Leder and his colleagues at NIH, details of the strain have already been published in *Nature*, and the committee members were provided with test data several weeks before their meeting. They certified the strain as being sufficiently weakened to provide an adequate biological safety barrier.

The second crippled micro-organism under consideration was a strain of the human gut bacterium *E. coli*, developed by Dr Roy Curtiss at the University of Alabama. Curtiss has been working on the strain for more than a year, and he has built a number of ingenious mutations into the organism to reduce its ability to survive outside the laboratory. He has also conducted exhaustive tests to measure the bacterium's chances of surviving. His test data were presented to the committee on the first day of the meeting in the form of a 147-page set of figures, graphs, tables and text. Though few members of the committee had the chance to go through Curtiss's data, they came close to voting on whether or not to approve the strain. Fortunately, however, it was decided—much to Curtiss's relief, it should be noted—to defer a decision for a month to allow committee members to consider the data and confer with other experts.

Though many members of the committee have been keeping in close touch with Curtiss's experiments over the past year, and were therefore well versed in his overall results, if the committee had certified his strain on the basis of such a cursory examination of the data, it would have laid itself wide open to criticism. Biological containment, after all, is a central feature of the safety guidelines. Nevertheless, Curtiss's data is so extensive and by all accounts it provides such convincing evidence for the feebleness of his bacterial strain that the committee is very likely to approve the strain for use in genetic manipulation experiments.

Within the next few weeks, guidelines will thus be issued by NIH to govern that agency's support of genetic manipulation experiments, and the committee's certification of at least one crippled micro-organism has paved the way for a lifting of the embargo in the United States. □

CANADA

Mercury poisoning in Ontario

Are Canada's Indians now victims of Minamata disease? David Spurgeon reports from Ottawa

IN THE spring of 1970, the minister of energy and resources management of the province of Ontario announced that the fish in the English-Wabigoon river system contained high levels of methylmercury and were unfit for human consumption. The sequence of events since then has seen the disruption of the way of life of hundreds of Indians in the area, the closure of a prosperous recreational fishing lodge there, serious doubt cast on the capacity of governments to deal with such issues, and a human tragedy recalling the mercury poisonings at Minamata, Japan.

Dr Masazumi Harada of Kumamoto University in Japan, a neuropsychiatrist and expert on Minamata disease, noted neurological symptoms in many Indians he examined in the area. "There were 37 cases in which some type of sensory disturbance was found," he reported. *"Impairment of the visual field was found in 16 cases. Seven of these cases had asymmetric constriction and tunnel vision. This is very significant in Minamata disease."* (Harada's italics). He was a member of a Japanese delegation that included victims of Minamata disease which last September visited the Canadian settlements affected at White Dog and Grassy Narrows Reserves, near Kenora, in northern Ontario.

The energy and resources minister singled out a paper company's chemical manufacturing plant as the cause of the mercury contamination in the area. Over the previous eight years it was said to have dumped eight tons of methyl mercury into the river system seventy miles upstream.

The process in which the mercury was used has now been replaced, but the advice given by the Ontario government to anglers is to fish for fun only. All commercial fishing licences held by the Indians were revoked in 1970 by the government, which has since supplied them fish to eat from other lakes. This removed a major source of income for the Indians, but it did not stop them eating contaminated fish. And in Grassy Narrows Reserve, where there had never been much reliance on government welfare support, welfare payments are now the principal source of income.

On the government's role, the Canadian Broadcasting Corporation has referred in its programmes to reports that were ignored or never came to light. Newspapers point to a conflict of jurisdiction between federal and provincial governments.

The line taken by government medical men is revealed by Dr James Stopps, senior consultant on environmental health for the Ontario government, who says "to date no Indian has been diagnosed as having mercury poisoning. That doesn't mean no cases exist; it does mean that after five years we haven't been able to find one." Dr Harada, however, says "even a conservative estimate is that the effect of methylmercury among seven cases cannot be denied." Critics further maintain that the Government's tests were carried out during the mid-winter rather than at the height of the fish-eating season; that it did not test some of the people who ate the most fish (the guides); and that it noted only the mercury levels of the individuals, not their symptoms.

The latest chapter in the story is found in an interim report, published by the federal health department, on a survey of residents of the White Dog and Grassy Narrows Reserves. The purpose of the survey was to determine the extent of exposure to mercury and whether such exposure is causing adverse effects on health; the report which deals with samples of hair and blood obtained at the reserves last August, was written by Dr T. W. Clarkson, professor and director of the Centre in Environmental Health Sciences of the University of Rochester's medical school and a consultant to the Research Institute of the Hospital for Sick Children in Toronto.

"The results obtained to date (December 1975) indicate a wide range of mercury levels—from less than 5 parts per 10^9 to 330 parts per 10^9 in blood," the report said. "The exposure to mercury usually follows a yearly cycle with the highest intake in the summer. Adult males acting as guides at fishing camps have the highest intake of mercury. Five guides out of eleven that were sampled had blood levels in the range of 100 to 330 parts per 10^9. Analysis of hair samples from the guide having the highest blood concentration (330 parts per 10^9) revealed that the peak mercury concentration had occurred two months prior to the collection of the blood sample. This peak value in hair was close to 150 parts per million, equivalent to a blood concentration of approximately 500 parts per 10^9".

The report also revealed that wives of guides tended to have higher blood levels of mercury than other females on the reserve. Children usually had lower levels than their parents, but younger children tended to have higher levels than older ones. "A matter of serious concern is the continued exposure of pregnant women to methylmercury," it said. By contrast, analyses of blood samples received from women (mostly non-Indians) living in or around nearby Kenora, and received from the Lake of the Woods Hospital, Kenora, revealed no significant exposure to methylmercury at the time of the delivery of their babies.

Picture: Mansell Collection

A thorough clinical evaluation of the health of those sampled will not appear until phase two of the study. But the report "tentatively" concluded that "several individuals in both reserves still have unacceptably high blood levels of methylmercury . . . very high exposure to methylmercury continues to be a serious problem with guides working at fishing camps." It noted that the highest concentrations reported occurred in the summer of 1975 "despite the fact that warnings against eating contaminated fish have been in effect for several years and that an intensive health education campaign has been in effect during the past year."

The report recommended further action by the guides' employers and health authorities to reduce exposure, and high priority for the planned expansion of a clinical programme. It recommended continuation of the health education programme and—because "the mercury problem may continue for some time"—establishment of a National Centre in Environmental Health. Such a centre, the report said, could deal with other environmental pollutants as well as methylmercury, and "would help detect and deal with potential threats long before they emerged as a national problem." ☐

SOLAR ENERGY

Power from the sun

Pierre Langereux of La Recherche *reports from France following an international symposium on solar electricity held last month in Toulouse*

"BEFORE solar energy becomes a major energy source for the 21st century, its development will require financial expenditure of the same order of magnitude as that needed for the development of nuclear energy." So said the scientific director of the National Scientific Research Centre (CNRS), which, together with the National Space Research Centre (CNES), organised the first international colloquium on solar electricity. He was speaking at the end of the meeting, which was attended by about 300 scientists from over 30 countries and produced an agreement on scientific and technical collaboration in developing solar energy through photovoltaic and thermodynamic conversion.

Photovoltaic conversion, perfected 20 years ago by Bell Laboratories and in general use since then to feed man-made satellites, is based on the capacity of certain semi-conductors (silicon, cadmium suphide, cadmium telluride, gallium arsenide) to transform light energy directly into electricity. Photopiles were developed for use in space exploration, where cost is of comparatively little importance, but they are still much too expensive to be widely used on earth. Silicon photopiles—the only ones currently in commercial use—have been used on earth for about fifteen years already, but for a few limited and highly specialised uses only.

In spite of the promising beginnings, there is still much to be done to make solar energy competitive with the other energy sources already in use. World production of photopiles for terrestrial application is currently equivalent to about 150 KW per year—100 KW in the USA, 30 KW in France, and the rest in Japan and Germany. But the market is expanding. In 1980 the production of silicon photopiles is expected to be of the order of about 10 MW: the price per watt of solar cells (currently \$21) may have been reduced by then to \$5 or \$10 through improvements in the manufacturing process, while in ten years the price could have fallen by a factor of 100 if current research on silicon cells in thin layers bears fruit. The most interesting prospects, however, seem to lie in the development of photopiles using other materials such as cadmium sulphide and cadmium telluride, which were prematurely abandoned for space projects several years ago.

What of thermodynamic conversion? This uses solar ray collectors to heat water which, when transformed into steam, activates an alternator or turbine after passing through a heat exchanger. To reach high power levels, solar towers are needed; heliostats receive solar rays and concentrate them onto a steam generator placed at the top of a concrete tower. The higher the tower, the greater is the capacity of the power station.

Thermodynamic power stations have evolved rapidly, especially in France, and the Sofretes company has recently installed the first solar thermodynamic power station at La Pas (Mexico). Other projects will follow, with more powerful experimental power stations—one of 1 MW at Odeillo (France), for example, and another of 5 MW in the USA. Both countries also have projects for building 10 MW solar power stations, while in Japan, Mitsubishi Heavy Industries has announced a plan for a 1000 MW solar power station.

Whichever of the two methods is envisaged for the future, however, the most difficult (and perhaps obvious) problem is yet to be resolved: how to store the energy produced by solar power stations, for they can only function when the weather is clear and sunny. In the photovoltaic system, electrochemical storage is expensive; with thermodynamic power stations, on the other hand, one can more easily use thermal storage. Material costs, which have already been studied for nuclear programmes, would be relatively acceptable, and three materials are being considered: oils, rocks and dissolved salts. Great hopes are based also on the possibility of stock-piling hydrogen, obtained as a by-product in the photodissociation of water.

The USA has embarked on a very ambitious programme, with the objective of supplying 5–7% of its energy needs from solar energy in the year 2000, and 25% in the year 2025. The Energy Research and Development Agency (ERDA) will assign \$120 million to the development of solar energy this year, and \$160 million in 1977. France, coming second, has set aside for this year a sum of the same proportionate size relative to the GNP—60–70 million francs, twice as much as in 1975. In comparison, Germany plans to spend only 36 million francs this year, and Japan slightly less. ☐

INFLUENZA

How it should work

THE House Appropriations Committee has unanimously approved the \$135 million requested by President Ford to inoculate the American public against a possible epidemic of 'swine' influenza next winter. The first batches of vaccine against this new strain should be ready in mid-April for clinical trials of its protective value.

If these are successful vaccination of those most at risk should begin in late summer. Representatives of the drug industry and government health officials testified before Congress this week that they hope to have the whole population inoculated by Christmas.

Rapid production of the vaccine can be achieved by an ingenious trick devised in the late 1960s. A quick-growing "master" strain of flu virus is hybridised with the swine flu to produce a recombinant bearing key characteristics of the swine flu virus. This recombinant can grow rapidly in fertilised hen's eggs, allowing vaccine production in 6-8 weeks rather than several months. The virus is then collected, killed and packaged into vaccine. It is reckoned that 50 to 100 million hen's eggs will be needed to produce the 200 million shots needed to vaccinate Americans. The protection expected with this vaccine will be between 70 and 80%.

The \$135 million will cover the costs of production and distribution of vaccine only. Vaccination will be voluntary and those who can will have to pay for their flu shots, but President Ford has stated that nobody will be denied vaccination if he cannot pay for it. The high-risk groups in the population will also automatically receive inoculation against the A and B strains which have been around this winter. But the emergency vaccine itself will only confer protection against the swine flu virus, which on its present showing in the one outbreak so far appears to produce generally less severe illness than the other strains prevalent this winter.

Meanwhile the Canadian government has also decided to approve a mass inoculation programme with swine flu vaccine. It plans to produce about 12 million doses, enough for about half the population, with preference to be given to high-risk groups.

IN BRIEF

Safeguards surface

A few days before taking office as Britain's new Prime Minister, Mr James Callaghan, speaking as Foreign Secretary, revealed in reply to a question in the House of Commons details of the safeguards Britain would apply in deals involving the transfer of sensitive nuclear technology. The safeguards, agreed among the (soon to be enlarged) Group of Seven nuclear exporting countries, demand assurances that the exported technology will not be used to make nuclear explosives for any purpose, that there is sufficient protection against the risk of theft or sabotage and that similar safeguards will apply both to re-export of the technology by the recipient and, for twenty years, to independent replication of nuclear plant by the recipient.

Deadline passed

The United States and the Soviet Union are to continue discussions which might yield a bilateral treaty on nuclear explosions even though they failed last week to meet the March 31 deadline originally set for agreement. The talks began in July 1974, when Mr Brezhnev and President Nixon agreed in principle that peaceful and military explosions should be limited to 150 kilotons and that there should be some on-site inspection. The remaining difficulty chiefly concerns monitoring multiple peaceful explosions which together exceed 150 kilotons that the USSR might wish to use in civilian projects.

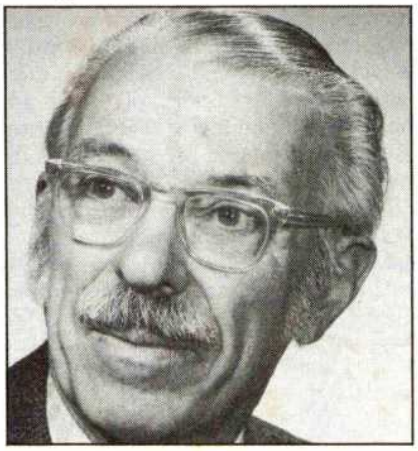
Asbestos inquiry

The renewed controversy in Britain over the health risks of asbestos to the public at large as well as to employees in the industry itself has led to the appointment of a government committee to make a wide-ranging review of the hazards involved and of the safeguards in use. The formation of the committee, to be headed by the chairman of the Health and Safety Commission, has been welcomed by the industry's Asbestos Information Council, and follows the publication of a report by the Ombudsman criticising the Factory Inspectorate's policing of a plant where 40 people died from asbestosis between 1939 and 1970.

UNEP plea

The annual governing council of the United Nations Environment Programme (UNEP), on which 58 states are represented, opened in Nairobi last week with a plea from its executive director, the biologist Dr Mustafa Tolba, that member states fulfil their pledges in order to resolve UNEP's financial crisis and so prevent it curtailing its plans. Only a small proportion of the money pledged has actually been received, with the big countries proving to be the most reluctant contributors.

Apricot retaliation



THOMAS H. JUKES

SPRING in California used to be signalled by a procession of fruit blossoms in the well-kept orchards: almonds in February, then apricots, sweet cherries, peaches, and, finally, the miles of prune trees in the Santa Clara valley that have now been displaced by houses. Most orchards have retreated into the background of the concrete invasion. In a few months, the fruit harvest will start with the sweet cherries, and later the wooden lugs of golden apricots will be loaded for shipment.

As a good Darwinian, I feel sure that the sweet and delectable flavour of apricots, enhanced by plant breeding, is meant to encourage me to pick the fruit and walk away with it. Then I shall devour it, and drop the stony pit on the ground so that the apricot will extend its territory when the seed germinates. But the seed doesn't want to be eaten, for this will destroy the embryo. So, in the seed, there is a substance called amygdalin that decomposes when the seed is macerated. This process liberates an enzyme, beta glycosidase, that hydrolyzes amygdalin to glucose and mandelonitrile, a compound of cyanide and benzaldehyde which breaks down to set free HCN. Death to eaters of apricot seeds! Having summarily disposed of its enemies, the apricot will take over the landscape.

The same game-plan is a hallmark of other members of the rose family, and, indeed, of many other plants—sorghums, lima beans, cassava, clovers and acacias. Almonds and beans have been selected by plant breeders for low content of cyanide. Cassava is soaked, fermented and leached to remove HCN. Until recently apricot pits were discarded, except occasionally the seeds were crushed and added to preserves to impart the almond flavour of benzaldehyde. The HCN presumably diffused away during cooking.

A new interest in apricot pits has come about as a result of the "health food" sponsorship of "Laetrile" (amygdalin) for the ineffective treatment of cancer. Dressed up as vitamin B_{17}, which is a completely unjustified soubriquet, Laetrile has become the darling of the diet stores. As a result, the apricot has struck back. Last October, a 34-year-old man residing in San Diego, California, developed symptoms of cyanide poisoning after eating apricot kernels. A package of the raw kernels was bought at a health food store and made into milk shakes, following a recipe in an "organic food" publication. The man's wife didn't like the taste, so her husband greedily downed both portions, and, one hour later, "developed forceful vomiting, headache, flushing, heavy perspiration, dizziness and faintness". He was rushed to the emergency room of the local hospital, and rescued by a stiff dose of ipecac. Once again, the process of natural selection was thwarted by human intervention.

The state health department drily states in its report "the minimal number of apricot kernels necessary to cause symptoms or death in humans is not known. Since this and similar products are widely distributed in health food stores for reputed nutritional and medicinal value, the possibility of disease from a large number of kernels should be recognised".

As you can guess, my apparently inhuman sympathies are with the plant world. I treat stinging nettles with respect, and I delight in the blue flowers of larkspur and monkshood. Unlike Bret Harte's "mliss", I do not plan to eat the leaves thereof. I disagree with D. H. Lawrence's idea that the devil himself must have conceived the spines of cacti in a moment of sheer ecstasy. Framed in thorns the exquisite blossoms of the cactus are to look at, not to touch. Plants have every right to tell us to keep our distance. *Hommage à Darwin!*

correspondence

Genetics at the OU

SIR,—The letter by Kacser *et al.* (April 1, page 386) concerning the Joint Universities Genetics Course, raises issues on which formal consultations between the Open University and the Nuffield Foundation are taking place. It contains statements that are false, misleading, and prejudicial to Open University students and it is my duty to comment.

Firstly it should be understood that the grant from the foundation is less than half of the costs of production of a half-credit second-level course, the rest being met by the Open University. Nuffield support, whilst generous, was essentially given to carry through a project devised and planned by the participating universities.

We have always had draft material assessed for its academic validity by independent experts before final publication; the genetics course is no exception; all its units have been independently assessed and developmentally tested with volunteer student panels.

Relatively late in the planning of the course, the Nuffield Foundation proposed, and collaborating universities agreed on, a Consultative Committee to aid in course development. The Committee's unanimously agreed rôle was "to advise and comment on the general course outline, aims and objectives" and "to provide a group of geneticists willing to serve as academic referees for particular detailed portions of the course". It had neither control of nor responsibility for the course or the use made of it in individual institutions. In the main this is how the committee has interpreted its rôle and this has been of great assistance to the course team. Inter-university collaboration is a delicate and difficult plant to nurture, and it is a pity that the harmony between different universities reflected in the work of the team has not carried through to all the members of the consultative committee.

Kacser *et al.* state that "the Committee informed the Foundation that it was dissatisfied with the outcome of the previous three years' work and asked that the course should not be published, or even used by Open University students, without further consideration, restructuring and amendment". This is untrue. A document signed by some members of the Committee (whose membership is incorrectly given in the letter), which was neither seen nor discussed at any meeting of the committee, and whose very existence was unknown to some of its members, was passed to the Director of the Nuffield Foundation.

Kacser *et al.* imply that the committee thought that the length and depth of the course was far beyond the capacities of the intended students. They state (as if they had only just discovered the fact) that, for Open University students, the study time for the average "Unit" is 12 hours, and that this work must be completed, on average, in a week. All this is, of course, perfectly true. But they then go on to assert that "it was the Committee's considered opinion" (though the Committee has never considered such an opinion) "that this is an impossible task if understanding at a University level is aimed at".

This workload is normal for all Open University courses. If the opinion of Kacser *et al.* is correct, the fact that many thousand students have successfully performed this "impossible task" over the past five years could only mean that these students did not achieve "understanding at a University level". This is a gratuitous insult to students and graduates and a grave reflection on our academic staff and external examiners.

Further, as Kacser *et al.* know full well, developmental testing of the course's components has continued this year with actual students. To pronounce upon the alleged difficulty and overloading of the course in advance of the completion of this evaluation seems to indicate prejudice rather than a spirit of objective criticism. To do so publicly seems to me to be irresponsible. Based on the first returns we have had from students, the average times taken to study Units 1 and 2 were 7 hours and 10 hours respectively, 99% of students reported on Unit 1 as being "OK" to "Very Interesting" and 98% found it "OK" to "Very Easy" to understand. The comparable figures for Unit 2 were 78% in each case. It would be premature to generalise from the first two units, but the facts so far contradict Kacser *et al's* mere opinion. I am confident that when the evaluation of the remaining Units—and the examiners' assessment of students' performance—has been completed, Kacser *et al.* will be shown to have been wrong.

Kacser *et al.* further suggest that the use of this course by other institutions "has not been seriously considered". Quite the contrary: the other participating universities have, I believe, already made plans to use sections of it in their own teaching programmes and several other institutions have already let us know of their plans to do the same. It is also misleading to claim that an "obvious deficiency" of the course is that it has no "practical manual". At an early stage the team discussed practical work and decided that because of the widely differing circumstances of the different institutions that might use the course, no attempt should be made at present to prepare a manual except for OU students.

Kacser *et al.* assert that "the Consultative Committee jointly with the Nuffield Foundation suggested a number of changes which would have gone some way to meet these criticisms, but they were not accepted by Professor Rose". This is false in one respect and misleading in another. At no time has the "Consultative Committee jointly with the Nuffield Foundation" suggested these changes. Of course, in the design and development of the course, comments from members of the committee have been submitted to the team; many of these were accepted, with appreciation. The statement misleadingly suggests that acceptance or otherwise of comments was the prerogative of Professor Rose. In fact, all such decisions were taken by the full course team.

I very much regret the inaccuracy and tone of the letter, and wish to reiterate my belief that this course is academically and educationally on a par with other second-level Open University science courses, the value and success of which is already well established. Because of the wide interest in the subject and the way in which the course is constructed, the course will be of use in many other teaching contexts than the Open University.

Yours faithfully,

M. J. PENTZ

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news and views

Ectopic production of HCG subunits

from a Correspondent

THE synthesis of a particular hormone or hormone subunit by tumours derived from a tissue not normally engaged in its production (ectopic production) is now a familiar observation. Endocrine abnormalities in patients can often be traced to the presence of a tumour in non-endocrine tissue. Measurement of raised levels of some hormones circulating in the blood is often used as a biochemical marker of malignancy and to monitor the success of surgery, analogous for example to the use of chorionic gonadotropin assays in women with choriocarcinoma. Human chorionic gonadotropin (choriogonadotropin) (HCG) is synthesised by the trophoblast at a very early stage of pregnancy—within a few days of fertilisation. It provides a critical support to the corpus luteum, which decays in its absence with resulting menstruation, and so is crucial in establishing and maintaining pregnancy. HCG is not normally present in the circulation of non-pregnant females, the only exceptions being patients with choriocarcinoma (Vaitukaitis *et al.*, *Ann J. Obstet. Gynecol*, **113**, 751–758; 1972), hydatidiform mole (Crawford, *Brit. Med J.*, **4**, 715–719; 1972) and patients with cancers that ectopically synthesise the hormone (Braunstein *et al*, *J. clin. Endocrinol. Metab.*, **35**, 857–862; 1972; Braunstein *et al.*, *Ann intern. Med.*, **78**, 39–45; 1973). HCG can also be detected in normal testis (Braunstein *et al.*, *New Engl J. Med.*, **293**, 1339; 1975).

The ectopic production of chorionic gonadotropin in cell lines was first described in Gha-Go cells isolated from a bronchogenic carcinoma (Rabson *et al*, *J. natn. Cancer Inst.*, **50**, 669–674; 1973; Tashjian *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1419–1422; 1973). Both subunits of HCG were identified. The α subunit of HCG (HCG-α) is nearly identical to the α subunits of thyroid-stimulating hormone (thyrotropin), follicle-stimulating hormone (follitropin) and luteinising hormone (lutropin) whereas the β subunit (HCG-β) is different from the β subunits of the other glycoprotein hormones. The α and β subunits are glycoproteins containing moderate amounts (10–20%) of carbohydrate. Two papers in *Nature* have now shown that these subunits are synthesised and secreted by HeLa cells in culture.

A hundred years ago

ON the 31st ult. a meeting, at which several well-known English biologists were present, took place at the house of Dr. Burdon-Sanderson, at which the advisability of establishing a society or association for the purpose of promoting the progress of physiological research in England, was considered and discussed.

from *Nature*, **13**, April 6, 455; 1876

Ghosh and Cox (*Nature*, **259**, 416–417; 1976) used the HeLa 65 and HeLa 71 sublines. They showed by radioimmunoassay with β subunit antiserum that the secretion of small but significant amounts (~ 0.0024 ng per 10^6 cells over 5 d) of immunologically reactive material occurs during culture. It is not clear from the results of Ghosh and Cox whether β subunit production, secretion of the complete hormone or both processes takes place. Furthermore, there is a crucial question of the degree of specificity of antiserum for β subunit, as considered later. Perhaps the most striking result reported by Ghosh and Cox is the greatly increased (1,000-fold or more) secretion of immunologically reactive material when HeLa cells were grown in medium supplemented with 1–5 mM butyrate. The response of the cells to butyrate varied almost linearly up to 20 ng per 10^6 cells over 5 d with butyrate concentration over the range 0–5 mM. This intriguing finding is apparently not specific to HCG production. The levels of activity of placental alkaline phosphatase, which like HCG is produced ectopically by HeLa cells (Elson and Cox, *Biochem. Genet.*, **3**, 549–561; 1969) are also markedly increased by butyrate (Griffin *et al.*, *Arch. Biochem. Biophys.*, **164**, 619–623; 1974) The mechanism of action of butyrate in augmenting synthesis of two glycoproteins is unknown. It may be significant that short chain fatty acids including butyrate, were shown to increase the activity of a sialyl transferase in HeLa cells (Fishman *et al.*, *Biochim. biophys. Acta*, **59**, 292–299; 1974), since sialylation of at least one glycoprotein, thyroglobulin, seems to be obligatory for its secretion (Monaco and Robbins, *J. biol. Chem.*, **248**, 2328–2336; 1973). The substrate specificity of the HeLa cell sialyl transferase(s) responding to butyrate may therefore be of interest in considering its possible role in controlling HCG secretion.

Although the Ghosh and Cox experiments do not distinguish between secretion of β subunit or completed HCG molecules, Lieblich *et al.* (this issue of *Nature*, page 530) show unequivocally that at least some sublines of HeLa secrete relatively massive quantities of α subunit ectopically (about 75 ng per 10^6 cells per day). Unfortunately the HeLa sublines (CCL 2, 2.1 and 2.2) used by Lieblich *et al.*, do not include those studied by Ghosh and Cox. Since the rate of secretion of α subunit varies about 50-fold among the various sublines studied by Lieblich *et al.*, it is possible that HeLa 65 in fact secretes only the β subunit. Alternatively these cells may carry out a balanced synthesis of α and β subunits with secretion of the completed hormone.

The relatively massive secretion of free α subunit by the HeLa sublines studied by Lieblich *et al.*, does however raise the question of what Ghosh and Cox were measuring in their basal system or even in the stimulated cultures. The antiserum used by the latter workers, raised against purified β subunit, is stated to show less than 1% cross reactivity with luteinising hormone indicating even less than this degree of cross reactivity with the common subunit. But such contamination could conceivably account for the small reactivity obtained in the medium of cells grown in the absence of butyrate. It is of course less likely to complicate the measurement of the much larger quantities of immunologically reactive material produced by HeLa 65 cells in butyrate medium although this cannot be entirely ruled out.

The α subunit secreted in such large amounts by HeLa cells is immunologically indistinguishable from α subunit isolated from pregnancy urine. The tumour α subunit is apparently not structurally identical however, since Lieblich *et al.*, found a small but definite difference in elution of the tumour α subunit during gel filtration compared with the pregnancy subunit. A slightly increased molecular weight could account for this discrepancy, although glycoproteins often behave abnormally during gel filtration chromatography. Weintraub *et al.* (*J. clin. Invest.*, **56**, 1043–1052; 1975) showed similar abnormal properties for α subunits secreted by the Gha-Go cell line. In gel chromatography the Gha-Go subunit behaved as a protein of 27,000–30,000 apparent molecular weight, significantly higher than the α subunit found in pregnancy (23,000–24,000 apparent molecular weight). Thus, there seems to be an increase in the size of α subunit made by tumours. It may be that this material represents a precursor form present only transiently in the production of normal α subunit and HCG by placental cells. Interestingly, the Gha-Go tumour α subunit could not form biologically active HCG when incubated with HCG β subunit and tested in a rat testis radioreceptor assay. By contrast HCG α subunit from pregnancy urine combined efficiently with HCG β subunit to reconstitute 95% or more of the expected HCG activity under the same conditions. It seems therefore that tumour α subunits may be defective in a stage leading to functional HCG molecules. The molecular basis for the difference is presently unknown. It is possible that the α-subunits are degraded in some way by tumour-specific proteases, although this seems unlikely from their apparently greater molecular size compared with the subunit of pregnancy urine. Their amino acid compositions are also very similar to pregnancy α subunit except for small differences in some residues, particularly valine, phenylalanine and half cystine. Perhaps further analysis by peptide mapping or comparison of carbohydrate contents might suggest the structural differences between biologically active α subunit and the apparently inactive product of tumour cells. □

Why don't animals cheat?

from John Krebs

As a general rule, animals settle disputes with other members of the species by means of ritualised displays rather than by physical violence. In contests for food, territories and mates, raised crests and songs serve as substitutes for teeth and claws, and the loser retreats unscathed. This much is well known, but it is less clear why the ritualised communication system does not break down because of cheating. It is easy to imagine that the winner of a ritualised fight could save itself the risk of future harassment by killing the loser, yet even animals with powerful jaws or horns rarely do this; instead they play by the rules. Similarly, if a dominant individual claims a territory by, for example, loud song, why do submissive individuals not bluff their way to success by singing?

The first attempt at a formal theoretical analysis of these problems was made a few years ago by John Maynard Smith (*Nature*, **246**, 15; 1973; *On Evolution*, Oliver and Boyd, 1972). Using game theory, Maynard Smith was able to analyse why physical violence (which he calls escalated fighting) does not replace the 'conventional' system of ritualised signals. His method of analysis was to look for an evolutionarily stable strategy, or ESS. An ESS is defined as a strategy which, when it is used by the majority of individuals in a population cannot be bettered by any alternative rare 'mutant' strategy. The 'strategies' which Maynard Smith considered included such alternatives as 'hawk'—an individual which always escalates—and 'mouse', which only plays the conventional game and retreats at the first sign of violence. He then assigned payoffs for winning, losing, getting injured in an escalated fight and so on, and used these values to construct a payoff matrix for each strategy against all others, including itself. It is intuitively clear that the hawk strategy does very well against a mouse; but does poorly in a population made up mainly of other hawks, because the risk of injury is high, so it is not an ESS. Only in the extreme case where losing a contest means certain death, say from starvation, will hawk persist as the best strategy. Otherwise the most likely ESS, for a wide variety of payoff values, was a mixed strategy of mainly ritualised display, but with escalation in retaliation to violence by an opponent. Fortunately, this seems to be the common pattern of contest in real animals. In another model Maynard Smith (*J. theor Biol.*, **47**, 209; 1974) showed that if a species has no method of escalating (because it does not possess dangerous weapons) so that the main cost involved in a contest is time, the ESS is again a mixed one with the distribution of persistence times given by the expression $(1/v)\exp(-x/v)$ where v is the value of winning and x the persistence time.

In a recent paper, Maynard Smith and Parker (*Anim. Behav.*, **24**, 159; 1976) have taken the analysis a step further by considering combats in which there is an asymmetry. In the real world two contestants will rarely be identical; one may be larger than the other, or may have more to lose, say because it has invested effort beforehand in establishing a territory.

In the latter case, where the payoffs are unequal, it is an ESS for the individual with more to lose (the territory holder for example) to invest more in the contest. Although there is a 'paradoxical' ESS in which the intruder invests more, Maynard Smith and Parker show that this ESS is unlikely to occur in nature, because, unlike the common sense ESS, the 'paradoxical' strategy cannot invade a hypothetical ancestral population which ignores the asymmetry. In the other type of asymmetry, where differences such as size give an indication of the probable outcome of an escalated fight, the ESS depends on how accurately each individual assesses its own size in relation to that of the opponent, and how good size is as an indicator of the outcome of a fight. It turns out that size, or some other such cue, will be used as a conventional means of settling a contest if the contestants estimate it with certainty, even if it is a poor predictor of the outcome of a fight. The ESS will be "always retreat if you think your opponent is larger, always escalate if you think he is smaller, and do something intermediate if he is the same size".

This automatically leads one to consider the question of bluffers. If it is an ESS for animals to use features such as size to settle asymmetric contests, can bluffers, individuals with the equivalent of padded shoulders, spread through the population? Maynard Smith and Parker conclude that bluff will always tend to spread but it is probably limited by the ability of other individuals to detect the difference between bluff and real fighting ability.

At the moment, this new approach to analysing ritualised contests is still in the theoretical stage, but some data seem to support the theory. Male dung flies (*Scatophaga stercoraria*) competing for females at newly deposited cowpats show the negative exponential distribution of persistence times predicted by the 'no escalation' model. Although this in itself is not a very surprising result, the fact that males with different persistence times have an equal chance of mating suggests that the distribution is in fact an ESS. •

Monocular deprivation and the input to the visual cortex

from A. M. Sillito

THE role of visual experience in establishing the functional properties of the developing mammalian visual system is a topic which has aroused considerable controversy and has been the subject of a recent review in *Nature* (Barlow, *Nature*, **258**, 199; 1975). There is however general agreement in this field that a normal input from the two eyes in early life is essential for maintaining an effective binocular input to visual cortical neurones. If monocular deprivation is produced in a kitten by suturing together the eye-lids of one eye at eye opening time for a period of two months or more it will be found that the majority of cortical neurones will be driven only by the non-deprived eye instead of both eyes as in a normal animal (Wiesel and Hubel, *J. Neurophysiol*, **26**, 1003; 1963). There is a critical period during which monocular deprivation is effective in producing these changes. In kittens it extends from some time in the third week of life to approximately three months and the effects of deprivation extending through the critical period seem to be irreversible despite subsequent long periods of normal binocular vision (Hubel and Wiesel, *J. Physiol.*, **206**, 419; 1970). Even quite brief periods of monocular deprivation during the first month of the critical period will result in the deprived eye's appearing to lose access to cortical cells and an essentially complete takeover by the non-deprived eye (Peck and Blakemore, *Expl Brain Res.*, **22**, 57; 1975). However, reverse suturing (opening deprived eye, suturing the normal) within the critical period can result in the previously deprived eye's regaining its ability to drive cortical cells and a complete reversal of the original situation can even occur in which all the cells are driven by the previously deprived eye (Blakemore and Van Sluyters, *J. Physiol.*, **237**, 195; 1974).

Of particular interest at present are the mechanisms contributing to the effects of monocular deprivation. Although there are morphological changes in the layers of the lateral geniculate body (the thalamic relay between the eye and visual cortex) receiving an input from the deprived eye the functional properties of these cells seem to be relatively unchanged (Wiesel and Hubel, *J. Neurophysiol.*, **26**, 987; 1963). In fact binocular deprivation produces similar morphological changes in the lateral geniculate body but does not result in a dramatic loss of binocularly-driven neurones (Hubel and Wiesel, *J Neurophysiol.*, **28**, 1029; 1965) This type of evidence together with the results of reverse suturing experiments carried out within the critical period have led to the view that there is a competitive interaction at the cortical level between the afferent terminals deriving from the respective eye inputs. It has been suggested that there may be an actual growth and reorganisation of afferent terminals (Movshon and Blakemore, *Nature*, **251**, 504; 1974) with the implication of a loss on cortical cells of some or all the terminals deriving from the deprived eye. Alternatively, it can be argued that during the critical period synapses that are used become 'more effective', whilst those that are not are in some way 'silenced' although still present, and that within the critical period this is a reversible process. A recent paper (Duffy, Snodgrass, Burchfiel and Conway, *Nature*, **260**, 256; 1976) has presented some important new evidence on this topic. They found, in cats monocularly deprived by lid suture from the fourth week of life to eight months, that intravenous injection of bicuculline revealed an input from the deprived eye to cells that were otherwise only driven by the normal eye. Thus they managed to reverse briefly the effects of monocular deprivation and furthermore they found that within this period, the receptive field properties in the two eyes were basically the same. The alkaloid bicuculline is an antagonist of γ-aminobutyric acid (GABA) in the central nervous system and GABA is a putative inhibitory transmitter in the retina, lateral geniculate body and visual cortex. Bicuculline applied iontophoretically to visual cortical neurones will produce changes in their receptive field properties that are consistent with a loss of an intracortical inhibitory input (Sillito, *J. Physiol.*, **250**, 305; 1975). Hence the results of Duffy *et al.* suggest that the deprived eye actually retains an input to cortical cells but that this is in some way suppressed by GABA-mediated inhibitory process. It is tempting to view this simply as a block of intracortical inhibition but the presence of GABA-mediated inhibition at other levels in the visual system means that intravenously injected bicuculline will produce changes in excitability throughout the system and this may contribute to the observed effect.

Whilst no one would dispute that monocular deprivation from the fourth week of life to the eighth month produces an irreversible loss of the ability to drive cortical cells from the deprived eye, it must be noted that in the experiments of Duffy *et al.* the deprivation started in the fourth week and not at eye-opening time. This means that the animals had approximately one week of binocular visual experience partially overlapping the beginning of the critical period. This is reflected by the similarity of the receptive fields in the deprived eye to those in the normal, an occurrence which if deprivation had been from eye-opening time might not be expected in so far as the receptive fields would be more likely to be of the immature type (Blakemore and Van Sluyters, *J. Physiol*, **237**, 195; 1975). It is important that the effects of intravenously injected bicuculline should be examined in a situation where monocular deprivation is started at eye-opening time, and hence where there will have been no opportunity for initial reinforcement of binocular input to cortical cells. Probably the most crucial experiment would be to examine the effects of iontophoretically applied bicuculline on visual cortical cells in monocularly deprived cats to see whether a local block of GABA-mediated intracortical inhibition can reveal an input from the deprived eye. With the present evidence in mind there seems little doubt that some type of inhibitory suppression of the input of the deprived eye, either with or without a concurrent loss of synapses or synaptic effectiveness, constitutes an important component of the cortical effect of monocular deprivation. ☐

T antigen and DNA synthesis

from Walter F. Mangel

POLYOMA and SV40 virus induce an intranuclear tumour (T) antigen early in lytic infection in transformed cells and in tumour-bearing animals. T antigen appears in lytically infected cells at the same time as the early virus-specific 19S mRNA and is followed by the induction of chromatin replication. This temporal sequence of events led to the hypothesis that T antigen is an early virus-coded protein which is responsible for the induction of cellular DNA synthesis that occurs after infection by papova viruses. In three recent articles from the laboratory of A. Graessmann in Berlin, direct experimental support consistent with this hypothesis is presented.

If T antigen is the early gene product

of polyoma, then microinjection of early polyoma RNA sequences into the cytoplasm of permissive cells might result in a positive immunofluorescence test for T antigen (Graessman *et al.*, *Nature*, **258**, 756; 1975). So polyoma DNA was symmetrically transcribed by the *Escherichia coli* DNA-dependent RNA polymerase. This complementary RNA (cRNA) contained both early and late viral sequences because the early virus-specific cytoplasmic RNA originates from 50% of the E strand of polyoma DNA and the late mRNA from 50% of the L strand (Kamen *et al.*, *Cold Spring Harb. Symp. quant. Biol.*, **39**, 187; 1974). On removing the DNA, the biological activity of the cRNA ($1\ \mathrm{mg\ ml^{-1}}$) was assayed after microinjection of about 10^{-11} ml into individual secondary mouse tissue culture cells Intranuclear T antigen was first detectable 6 h after cRNA microinjection in 6% of the cells Thereafter the number of T antigen-positive cells as well as the intensity of T antigen-specific fluorescence per cell increased, reaching a maximum of 70% of the cells between 20 and 24 h, before declining to undetectable levels 65 h after cRNA microinjection. V antigen (capsid protein) which is a late virus-coded protein (Mangel, Hewick and Smith, unpublished) appeared with the same kinetics as T antigen

Microinjection of asymmetrically transcribed SV40 cRNA which contains the early mRNA sequences not only results in T antigen formation but also in the induction of cellular DNA synthesis (Graessmann and Graessmann, *Proc natn. Acad. Sci. U.S.A.*, **73**, 366; 1976). Between 24 and 26 h after microinjection, 70–80% of the epithelioid cells of confluent primary mouse kidney cultures were T antigen positive. In pulse labelling experiments, the maximum proportion of cells synthesising DNA was reached between 15 and 20 h. During this time interval, 85% of the T antigen-positive cells were incorporating tritiated thymidine whereas only 3.2% of the mock injected cells in control experiments were synthesising DNA.

Important controls for these experiments included the demonstration that the biological activity of the cRNA was destroyed by RNase but not DNase. Although actinomycin D inhibited T antigen formation in mouse cells either infected with wild type polyoma virus or microinjected with polyoma DNA, it did not reduce the cRNA-induced T antigen formation within the first 15 h. Inhibition of protein synthesis by cycloheximide, added to the cells after cRNA injection, prevented T antigen formation Finally, neither total RNA nor polyadenylated RNA isolated from uninfected cells induced T antigen synthesis on microinjection.

In a series of preliminary experiments to confirm that the early genes induce T antigen the four fragments of SV40 DNA produced by the two restriction endonucleases *Hpa*I and *Hpa*II were individually microinjected into monkey cells (Graessmann *et al.*, *FEBS Lett.*, **61**, 81; 1976). Only one fragment induced T antigen synthesis and none induced V antigen synthesis. The fragment, which corresponds to 36% of the total genome, contains only 60% of the early region and thus could code for a protein with a molecular weight of up to 60,000. This is the size of the largest polypeptide immunoprecipitable by T antibody that is synthesised in the wheat germ cell-free system using asymmetrically transcribed SV40 cRNA as message (Roberts *et al.*, *Proc. natn. Acad. Sci U.S.A.*, **72**, 1922; 1975). But with early SV40 mRNA as message in the wheat germ system, the largest immunoprecipitable polypeptide synthesised has a molecular weight of about 90.000, which implies that the entire early region codes for T antigen (Smith *et al.*, and Prives *et al.*, in *In Vitro Transcription and Translation of Viral Genomes* (edit. by Haenni, A., and Beaud, G.,) **47**, 331 and 305, Editions INSERM, Paris, 1975) The results of Tegtmeyer (*Cold Spring Harb. Symp. quant. Biol.*, **39**, 9; 1974) indicate that the *in vivo* T antigen has a molecular weight of about 90,000. Thus the polypeptide induced by the *Hpa*I and *Hpa*II fragment may only contain some antigenic sites for T antibody and be biologically inactive. It would be interesting to know whether this fragment can induce DNA synthesis and transform cells.

The microinjection experiments support the hypothesis that T antigen formation requires early viral RNA sequences which must be translated and that all cells synthesising T antigen within the first 25 h of SV40 cRNA microinjection are also stimulated to synthesise DNA. The results are consistent with T antigen being the early virus-coded protein. They do not, however, exclude the possibility that T antigen is a cellular protein which is antigenically modified by the cRNA translation product. To show that T antigen is entirely a virus-coded protein, asymmetrically-transcribed SV40 cRNA must be translated *in vitro* and the product's tryptic peptides proved to be identical with those from T antigen isolated from transformed cells. Once T antigen is purified, the microinjection technique may be used to determine whether this protein is both necessary and sufficient for the induction of DNA synthesis as well as for transformation, an experiment whose results are eagerly awaited. □

Microtextures and rock deformation

from K. R. McClay

The rheology of rock masses in the Earth's crust and upper mantle has been the object of intensive study over the past decade, encompassing phenomena from the formation of slaty cleavage and mylonite genesis, to peridotite deformation. Results of research on these topics from schools in France, Germany, the Netherlands, Belgium, Great Britain, Australia, and America were presented at the recent conference on Fabrics, Microtextures, and Microtectonics, held on March 3–5, 1976, at Leiden, the Netherlands. The conference was organised by the Department of Tectonics, Leiden University, and papers arising will be published in a special issue of *Tectonophysics* later this year.

THE aim of the conference was an assessment of the role of fabric, microtextural and microtectonic information in the interpretation of geological processes. Microtextures (often called microstructures) are the morphological characteristics of rocks, embracing features from microfolding and cleavages to dislocation substructures The recognition of a particular microtexture as being characteristic of a certain deformational and thermal environment, is fundamental in structural geology. Relict microtextures, overprinting relationships and microtextural transisitions are all used in the estimation of temperature, pressure and rate of deformation for a geological event.

Fabrics are the distribution in space of discontinuities in rock masses, (that is, preferred crystallographic orientations). Increased application of X-ray texture goniometry and of metallurgical theories has generated a new impetus in fabric studies. Model studies of fabric development indicate that it may be possible to use fabrics as an aid in the determination of the deformation environment, strain history and the kinematics of deformation.

For a considerable time, the study of fabrics and microtextures was essentially the collection of data with little interpretation It now seems possible that a number of fabrics and microtextures may be understood in terms of the kinematics of deformation —particularly the evolution of foliations, mylonites, and sheared peridotites.

The first part of the conference dealt

with materials amenable to metallurgical techniques, and it became clear that research emphasis has changed from finite strain studies to kinematics, with the realisation that the kinematics of the deformation history have an important role (for example B. Hobbs (Monash University, Australia), and A. Nicholas (University of Nantes, France)). Crystallographic preferred orientations may reflect the influence of deformation paths as clearly documented in the field studies of quartz mylonites by J-L. Bouchez (University of Nantes) and by J. Carreras, A. Estrada (University of Barcelona), and S. White (Imperial College London). It was shown that the quartz *c*-axis fabric is dependent upon the deformation path of the mylonite. The experiments on marble by E. Rutter and M Rusbridge (Imperial College) support the theoretical predictions of the influence of the deformation path on the development of preferred orientations—particularly the predictions (G. Lister, Leiden University) that the new preferred orientations overprint the old fabric patterns after approximately 30% strain.

Theoretical fabric analysis using a metallurgical approach is a most exciting development. Using a Taylor-Bishop-Hill model, Lister has computed *c*-axis preferred orientations for quartz polycrystals utilising various combinations of slip systems. This allows simulations of deformations at a number of metamorphic grades to be made and the computed fabrics agree well with those found in naturally deformed quartzites. A. Etchecopar (Montpellier University) has used a different approach to fabric modelling with a two-dimensional single slip analysis which gives agreement with specific quartz and peridotite shear fabrics. These models allow postulates about the deformation mechanisms and the strain history of the rock to be made. For the first time a clearer understanding of fabric development and its complexities is at hand. In conjunction with fabric models, G. Davies (University of Cambridge) analysed the preferred orientation development in a deformed metal and demonstrated that, with the aid of orientation distribution functions, it is possible to assess the relative roles of various deformation elements such as glide, twinning and phase transformations during fabric development. Orientation distribution analysis, coupled with theoretical modelling is a potentially powerful tool in understanding rock fabrics.

Dealing with recrystallisation processes during or after deformation of a metal, J Jones (McGill University, Canada) pointed out that the differentiation between dynamic recovery, cyclic, continuous, or annealing recrystallisation was of fundamental importance in the development of fabrics and microtextures. A study of the deformation behaviour of single crystals of galena at various strain rates and temperatures (K. McClay and B. Atkinson, Imperial College) indicated that dynamic recovery and recrystallisation processes in galena occur at surprisingly low temperatures (for example migration of kink band boundaries at 200 °C).

Other important concepts brought out included the role of the yield surface both in single crystals and in polycrystals in determining the response of the material to an applied stress or to an imposed strain. In addition, Burgers vector and glide plane determinations seem critical in allowing further advance in theoretical models for deformation and fabric development. S. White (Imperial College) pointed out that subgrains may have a memory of deformation conditions and discussed in detail the problems associated with the use of dislocation substructures in rocks in which a substantial amount of recovery has occurred during or after deformation. This difficulty was also discussed in the light of an extremely interesting report on thermal decoration of dislocations in peridotite nodules (Y. Gueguen, University of Nantes).

In the second part of the conference, contributions on the formation of foliations in highly anisotropic minerals were discussed. P. Williams (Leiden University) reviewed foliation development, emphasising the role of initial kinking of phyllosilicate in producing foliations and indicating that the foliation will reflect the XY plane of the finite strain ellipsoid during all stages of development only in special cases. Experimental contributions to the study of foliated rocks since 1960 were reviewed by W Means (State University of New York). His experiments using salt/mica analogues produced very good approximations to natural crenulation cleavages and foliations as defined by mica films in deformed sandstones.

An extremely interesting contribution in this section on highly anisotropic minerals was that of A. Maltman (University College of Wales, Aberystwyth). His experiments on soft sediment deformation produced structures simulating those found in zones of incipient slaty cleavage formation. R Knipe and S. White (Imperial College) reported on a detailed high voltage electron microscopic study of slates showing the change from a crenulated fabric in the low strain zones around a fold to a well developed slaty cleavage at higher strains. Excellent high magnification micrographs give us our first good look at the fine structures which can be resolved optically only with great difficulty. Evidence for the growth of new phyllosilicates during the crenulation process was clear. A study of glacier deformation by M. Hambrey (Geological Institute, ETH, Zurich) illustrated the complexities of foliation development.

It can be concluded that the increasing application of metallurgical techniques and theories in a kinematic approach to rock rheology and microtextural problems is a promising direction for research. In particular, certain quartz fabrics can be explained using a kinematic approach. There are also important changes in the interpretation of foliations and cleavages. Initial crenulation of an already existing bedding plane fabric seems to have an important role in cleavage generation. The relationship between the cleavage plane and the finite strain ellipsoid may not be important. In all, the emphasis has changed from finite strain analysis to studies of deformation mechanisms and kinematics in order to understand both the strain paths and deformational processes in geology. □

Mobile spaghetti

from P. V. E. McClintock

One of the central assumptions of Vinen's 19-year-old theory of the supercritical state of superfluid ^{4}He has been challenged by Ashton and Northby in a recent issue of *Physical Review Letters* (**35**, 1714; 1975) on the basis of their experiments at the University of Rhode Island.

The superfluid properties which liquid ^{4}He displays below its transition temperature at 2.18 K can only be maintained if the liquid is treated fairly gently. Large fluxes of heat or high velocities of flow can cause the superfluidity to disappear abruptly, taking the liquid into a so-called supercritical state. The nature of the supercritical state which results when a large thermal current is passed through the liquid formed the subject of Vinen's historic series of papers in the *Proceedings of the Royal Society* (**A240**, 114 and 128, 1957; **A242**, 493; 1957; **243**, 400; 1958), and it is this topic which has again been under study, using a different experimental technique, at Rhode Island.

Below its transition temperature liquid ^{4}He behaves as though it were an intimate mixture of two interpenetrating fluids: a superfluid component, with zero viscosity, able to indulge in non-dissipative flow at low

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velocities; and a normal fluid component which has ordinary liquid properties and carries all the thermal energy. For small thermal fluxes, the liquid is almost a superconductor of heat, its being virtually impossible to set up a significant temperature gradient. This is because of a very efficient internal convection mechanism, whereby there is a flow of superfluid towards the heater, where it is converted into normal fluid and then flows away again through the oncoming superfluid, carrying heat towards the cold end of the apparatus. There is thus a counterflow of the two components without any net movement of the liquid. As the heat input is increased, however, a critical level is eventually reached at which the liquid undergoes a sudden transition to the supercritical state, and a finite temperature gradient spontaneously appears.

On the basis of a series of elegant experiments, Vinen was able to show that this discontinuous change in behaviour was a consequence of the appearance in the liquid of a tangled mass of vortex lines Just as in any other fluid, a vortex in helium consists of a narrow linear core region about which the fluid (for ^{4}He, only the superfluid component) rotates with a velocity inversely proportional to its radial distance from the centre: an interesting difference, however, is that the circulation, or "strength", of superfluid vortices in helium, being quantised, is always the same, which introduces enormous simplifications into any calculations of the supercritical state. According to Vinen, when the velocity of counterflow of the normal and superfluid components exceeds a certain critical value, vortex lines are generated and, by providing a dissipative mechanism, they enable a temperature gradient to be set up. In his model the vortex lines form an irregular tangle, with their cores being arranged somewhat like a bundle of spaghetti, but in continuous motion because each vortex exists in the flow patterns of all its neighbours and, indeed, of other parts of itself. It is assumed that vortex line is created at constant rate by the normal–superfluid counterflow, but that there also exists a mechanism by which the lines are destroyed. when two elements of line with opposite directions of circulation approach closely enough they can annihilate each other. Competition between the creation and annihilation mechanisms therefore results in an equilibrium length L_0 of vortex line per unit volume characterising the vorticity Vinen proposed that, in the equilibrium state, the tangle of lines was both homogenous and isotropic. The latter proposition also implied that the vorticity would, on average, be at rest relative to the superfluid so that, in a thermal counterflow experiment it would be moving towards the heater. What Ashton and Northby have now accomplished is the first satisfactory measurement of the direction and the velocity with which the equilibrium tangle of vortex lines moves relative to a laboratory frame of reference.

Their experimental apparatus, constructed from the insulating material Lucite, was in essence exceedingly simple. A heater was placed against the inside end wall of a 10 cm long channel of rectangular (0.5 cm × 1 cm) cross section, whose other end was left open to the helium bath in which the cell was immersed: by sufficiently energising the heater, the helium in the channel could thus be brought into the supercritical state, with a thermal counterflow of the normal and superfluid components occurring along the channel between heater and bath. At right angles to this counterflow, recessed in the side of the channel, was an arrangement for generating ions and directing them into the liquid, where some became attached to the vortex cores (there being an attractive force between an ion and a vortex arising from the kinetic energy of the rotating superfluid which the ion replaces). Any given ion, after being held like this for a while, was subsequently released as a result of the annihilation of the element of vortex line on which it had been trapped. An electric field was provided to draw freed ions to a pair of electrodes—one nearer the heater, the other nearer the bath—mounted on the opposite side of the channel from the ion generator. By noting whether trapped ions were being released upstream or downstream from the ion generator, it was possible to determine unambiguously in which direction the tangle of vortex lines was moving, and it was also possible to measure its drift velocity.

In the event, Ashton and Northby discovered that, quite contrary to the picture proposed by Vinen, the vorticity does not move with the superfluid component; furthermore, they have been able to show that it actually moves in the opposite direction, that is, in the same direction as the normal fluid component. The existence of a net relative velocity between superfluid and vortex lines implies that, to some extent at least, the vorticity is anisotropic.

These new results do not seem to be in conflict with other aspects of Vinen's model, but his theory is almost certainly going to need some significant modifications. The authors suggest a possible approach, but further experiments are clearly required before one can be sure they are right. □

No surprises for vitamin B_{12}

by Peter Newmark

It is always a bitter sweet experience when a highly sophisticated investigation does little more than confirm what lesser studies had already more or less demonstrated. The pleasure that is gained from confirming the predicted plot is countered by the disappointment at finding no unexpected twists, let alone a surprise ending. A good illustration of that principle is to be found in a recent study of transcobalamin II (TCII), the plasma protein that transports the cobalamins (vitamin B_{12}) between tissues (Schneider *et al.*, *J. clin. Invest*, **57**, 27; 1976).

For some years it has been known that most of the vitamin B_{12} in human blood is not attached to TCII but to the completely distinct transcobalamin I(TCI), one of a family of vitamin B_{12}-binding glycoproteins. Vitamin B_{12} attached to TCI is only slowly transferred into the tissues. If the vitamin is bound to TCII, however, it is rapidly cleared from the blood. This knowledge has been obtained with the aid of radioactive cobalt-labelled vitamin B_{12} and it has therefore been impossible to determine directly the fate of the transcobalamins, although tissue uptake of TCII has been indicated.

In 1972 Allen and Majerus succeeded in applying affinity chromatography to the isolation of the transcobalamins. As a consequence Allen and his colleagues have since been able to prepare radioactive iodine-labelled transcobalamins and to study their fate simultaneously with that of the labelled vitamin bound to them. When human TCI-vitamin B_{12} was injected intravenously into rabbits, clearance of both protein and vitamin was at about the same slow rate as that of labelled bovine serum albumin (Burger *et al.*, *J. biol. Chem.*, **250**, 7700 and 7707; 1975) unless the TCI was desialylated, when both protein and vitamin were extremely rapidly cleared into the liver in a manner remininscent of that described by Morell *et al.* (*J biol Chem.*, **246**, 1461; 1971) for various desialylated glycoproteins.

A different picture emerged when similar experiments were repeated with TCII-vitamin B_{12}'(Schneider *et al*, *J. clin. Invest*, **57**, 27; 1976). In the first place the native protein and its vitamin disappeared rapidly from circulation and second, their destination was widespread rather than being confined to the liver.

Since, initially at least, the transcobalamin moiety of the complexes always accompanied the vitamin, the information gleaned by having the protein independently labelled was not much more than had been known from studies of crude transcobalamins with labelled vitamin alone. A poor return, one might think, for having to produce, for labelling, transcobalamins purified several million-fold from several hundred litres of plasma.

More original, although also predicted, was the data obtained on the fate of the TCII following its uptake into tissues. After a 30-min time lag radioactive iodine appeared in the urine in rapidly increasing amounts and with a molecular weight of less than 1,000, indicating linkage to, at most, a small peptide. That can only mean that TCII had been rapidly degraded in the tissues. In contrast the vitamin was either retained in the tissues or returned to the blood stream, with little appearing in the urine.

Whereas it is easy to understand why the vitamin should be retained it is, as Schneider *et al.* point out, harder to explain the wasteful way in which TCII is destroyed in the process (compare transferrin). Various microorganisms appear to be able to release vitamin B$_{12}$ from TCII and it is difficult to see why cell surface enzymes could not have evolved to do the same.

Tissue uptake of transcobalamin–vitamin B$_{12}$ complexes probably involves pinocytosis, a process that threatens to come back into vogue in relation to receptor modulation (Raff, *Nature,* **259**, 265; 1976). Regardless of that there is little doubt that the transcobalamins are destined to receive more attention in the near future. In part that is thanks to the possibilities opened up by Allen's elegant techniques and in part it is motivated by despair. This year is the 50th anniversary of the discovery by Minot and Murphy (for which they were to share a Nobel prize with Whipple) that a diet of raw liver could control the previously fatal pernicious anaemia. But we are still at a loss to understand the biochemical basis for the prevention of that disease by vitamin B$_{12}$, the active component of liver. □

Central anomalies: why so strong?

from Peter J. Smith

WHEN marine magnetic anomalies were first analysed in detail in the early 1960s, it immediately became clear that their amplitudes decrease with distance from the corresponding oceanic ridge. Possible explanations for this phenomenon were not long in coming. For example, the magnetic constituents of the oceanic lithosphere newly formed along ridge axes may change chemically with time, giving rise to a gradual decrease of magnetisation. Another suggestion was that older igneous crust would be overlain by a thicker deposit of sediment, so the anomalies as measured at the ocean surface would be attenuated with respect to those observed above younger crust with less accumulated sediment.

But although these and some other processes, acting either individually or together, could possibly explain a gradual decrease in anomaly amplitude, they were apparently insufficient (at least in the form originally envisaged) to account for the precise form of decrease actually observed. For there is usually a particularly large reduction in amplitude immediately beyond the central anomaly followed by a much more gradual decrease outwards towards the continental margins. In the case of slow-spreading ridges (<30 mm yr^{-1} half-rate), for example, the central anomaly amplitude can be at least twice as high as those of near neighbours, although for faster-spreading ridges the difference is less marked.

More recently, near-bottom magnetic measurements have shown that not only does the central anomaly have a relatively high amplitude, there is often also a large-amplitude, short-wavelength (<15 km) anomaly within it. With slow-spreading ridges the latter is not generally observed separately at the water surface because of loss of resolution with distance from the ocean floor; the wider and narrower anomalies combine in such cases to give a very large central anomaly. Over fast-spreading ridges, on the other hand, the two anomalies can usually be clearly distinguished from each other.

One possible explanation of the relative strength of the wider central anomaly, first proposed by Matthews and Bath (*Geophys. J.*, **13**, 349; 1967), is that all magnetised oceanic crustal blocks except the central one are contaminated with material of opposing polarity, thereby reducing the strength of their respective anomalies. Harrison (*J. geophys Res.*, **73**, 2137; 1968), on the other hand, concluded that this phenomenon would be insufficient, suggesting that there must also be a 'demagnetising effect' outside the central region. Klitgord (*Earth planet. Sci. Lett*, **29**, 201; 1976) has now taken up the question of the demagnetising effect in the particular context of the narrower central anomaly. From a new analysis of both surface and near-bottom magnetic data from many different spreading ridges, she concludes that the narrower anomaly is due to a zone of high magnetisation coinciding with the region of most recent extrusion. The absolute strength of this magnetisation is regarded as that to be expected from newly-formed basalt recently quenched in seawater. Its large magnitude relative to that of the adjacent older basalt, however, is attributed to the fact that the latter rock has undergone low-temperature oxidation.

This is not the first time that oxidation has been seen as the cause of decreasing anomaly amplitudes. Klitgord's point, however, is that in the light of recent work oxidation can be invoked to explain not only the gradual reduction of anomaly amplitude away from a ridge but also the more sudden drop at the edge of the central anomaly. The recent work concerned is partly that of Butler (*J. geophys. Res.*, **78**, 6868; 1973) who concluded that small stable single-domain titanomagnetite grains become superparamagnetic when oxidised to titanomaghemite and thus lead to a greater reduction in magnetisation than the oxidation of pseudo-single-domain or multi-domain grains, and partly that of Watkins *et al.* (*Earth planet. Sci. Lett.*, **8**, 322; 1972) who found that fresh pillow basalt has a zone of fine grains immediately below the glassy outer rim.

The argument is that many of the fine grains in this zone will be single domains with high magnetisation, which means that they will contain a high percentage of a pillow's total magnetisation. A given amount of alteration in the zone will therefore have a much greater effect on the overall magnetisation than the same amount of alteration in the larger-grained pseudo-single-domain interior of the pillow. Moreover, being both fine-grained and close to the exterior, the zone will be much more susceptible than the interior to alteration in the first place. Rapid oxidation of the fine-grained zone is thus envisaged as the cause of the sudden amplitude drop outside the narrower central anomaly, whereas slower oxidation of the interior is seen as the cause of the more gradual amplitude decrease away from the ridge.

The widths of the narrow anomalies in the ridge systems studied by Klitgord range from 2 km to 10 km, which compare with several tens of kilometres for the main central anomalies. The magnetic relationship between the two anomalies never clearly emerges, however. At one stage Klitgord suggests that "the narrow magnetisation high probably does not account completely for the difference in amplitude between the central anomaly and the flanking anomalies", implying that it accounts for some, and perhaps even a high (but unspecified) proportion, of the difference. On the other hand, it is acknowledged that in some cases the narrow anomaly does not exist at all.

articles

Effects of drugs on the processes regulating the functional activity of brain 5-hydroxytryptamine

A. Richard Green & David G. Grahame-Smith*

5-Hydroxytryptamine (5-HT), seems to be involved in depression and perhaps schizophrenia. A behavioural test of 5-HT action in animals has been used to clarify the mechanism of its action and the effects of various drugs used clinically in affective disorders.

5-HYDROXYTRYPTAMIME (5-HT) is present in the cell bodies and varicosities of neurones in specific tracts in the brain[1,2]. Much is bound to synaptic vesicles[3] and there is good evidence that it is synthesised at the sites at which it is found. In view of the circumstantial evidence, and in the absence of evidence to the contrary, it is assumed here that it acts as an orthodox neurotransmitter (see ref. 4). It has been suggested that 5-HT is involved in the hypothalamic control of body temperature[5], the sleep–waking cycle[6], pain sensation[7], and sexual behaviour[4]. There has been discussion about its role in schizophrenia[8], affective disorders[4], and morphine addiction[4].

To understand the physiological role of 5-HT and the effects of drugs on those systems in which it is involved, it is essential to consider the regulation of 5-HT synthesis and release and how both of these are concerned in its functional activity. There are probably four factors involved in presynaptic regulation: synthesis, compartmentation, intraneuronal metabolism and reuptake. These four processes are presumably integrated to control the release of appropriate amounts of 5-HT from the presynaptic nerve terminal, as well as the amount acting at the postsynaptic site and the termination of its action. The postsynaptic factors involved in the action of 5-HT may be considered at two levels: the postsynaptic receptor site itself and the total postsynaptic response, which depends on the activity of other neurones and may be influenced by other transmitters or drugs affecting them. These mechanisms will now be discussed.

Availability of the 5-HT precursor tryptophan

An important factor controlling how much 5-HT is made in the brain is the availability of its precursor tryptophan, because in physiological conditions tryptophan 5-hydroxylase, the enzyme limiting the rate of 5-HT synthesis, is not saturated with tryptophan (for review see ref. 4).

Administration or deprivation of tryptophan will raise or lower brain 5-HT[9–11] and small changes in plasma tryptophan alter brain 5-HT content[12]. After food deprivation[13,14] or immobilisation[13] there is an increase in brain tryptophan concentration and increased brain 5-HT turnover. The rise in brain tryptophan is not accompanied by changes in other amino acids[15] and correlates not with total plasma tryptophan[13] but with the fraction of tryptophan which is not protein bound[16] (free or ultrafilterable[17]). Tagliamonte and coworkers have also shown a relationship between free plasma tryptophan and brain tryptophan concentrations[18,19] and further demonstrated that salicylate, which displaces tryptophan from its plasma albumin binding sites, increases brain tryptophan and 5-HT synthesis[20].

The relationship between starvation and brain tryptophan may be due to the increase in plasma unesterified fatty acids brought about by food deprivation[16]. Curzon and colleagues have shown that addition of unesterified fatty acids increases free tryptophan in human plasma *in vitro*. *In vivo*, drugs which alter plasma unesterified fatty acid (UFA) concentrations alter plasma free tryptophan in the same direction[22]. Wurtman's group have found a similar relationship between plasma UFA and free tryptophan[23] but dispute the importance of free tryptophan in determining brain tryptophan concentrations and the rate of brain 5-HT synthesis. It is clear, however, that many factors influence the transport of tryptophan into the brain. Insulin release[24], ingestion of carbohydrates[25], the relative concentration of tryptophan to other plasma neutral amino acids which compete with tryptophan for uptake[26], and starvation[15], all affect the amount of tryptophan in the brain. Free tryptophan concentration is clearly therefore only one determinant of brain 5-HT turnover.

Recent work has now shown that hydrocortisone decreases brain tryptophan[27] and 5-HT concentrations[27,28]. This is due to increased activity of the heptatic enzyme tryptophan pyrrolase[27,28], which metabolises tryptophan and causes a decrease in the concentrations of free and total plasma tryptophan[29] and results in decreased concentrations of tryptophan not only in brain but in liver also[29]. This indicates another peripheral mechanism altering the availability of tryptophan and therefore brain 5-HT turnover.

Little is known of the processes by which tryptophan passes from plasma into the brain although it has been suggested that these are subject to diurnal rhythms which are accompanied by changes in brain 5-HT metabolism[30]. Tryptophan transport across synaptosomal membrane has been studied[31,32] and it has been shown that uptake is stereospecific, temperature dependent and inhibited by metabolic inhibitors, and that there are low and high affinity uptake systems. Several other amino acids compete for transport and exchange diffusion occurs.

Central control of 5-HT synthesis

Glowinski and colleagues reported that 5-HT synthesis was reduced when the intraneuronal concentrations of 5-HT were increased[33–35] by monoamine oxidase inhibition. These results and their interpretation are the matter of some dispute[36,37].

Chlorimipramine which inhibits the reuptake of 5-HT at the nerve ending[38,39] decreases the rate of 5-HT synthesis[40,41]. Since inhibition of reuptake potentiates the effect of 5-HT on 5-HT receptors[42,43] it may be that increased stimulation of 5-HT receptors causes inhibition of synthesis by a feedback mechanism. Modigh demonstrated that tryptophan hydroxylation was inhibited following chlorimipramine, possibly through an action on nerve impulse flow[44] in accord with observations that the hydroxylase is influenced by nerve impulse flow[45,46]. It has been shown that increased receptor stimulation does inhibit impulse flow in central 5-HT neurones[47]. Since imipramine

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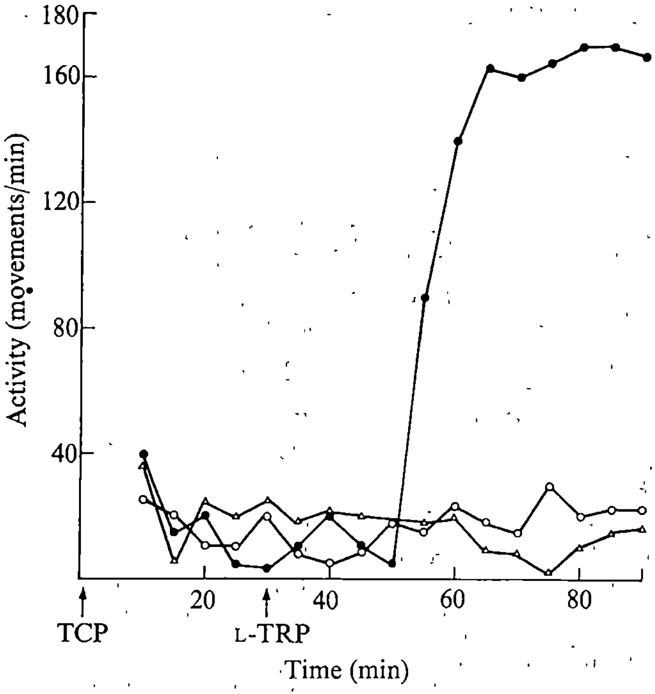

Fig. 1 Typical hyperactivity response in rats after administration of tranylcypromine (20 mg kg^{-1}) followed 30 min later by L-tryptophan (100 mg kg^{-1}) (●). Tranylcypromine administration only (○). Pretreatment with p-chlorophenylalanine (300 mg kg^{-1}) on two consecutive days before administration of tranylcypromine and tryptophan (△). Experimental details given in ref. 49.

inhibits tryptophan uptake by synaptosomes[48], however, some direct effects of chlorimipramine on 5-HT synthesis cannot be ruled out.

Functional activity of brain 5-HT

Our work was aimed at an understanding of how brain tryptophan concentration might control the rate of 5-HT synthesis and its functional activity. When L-tryptophan is administered to a rat there is a small increase in brain 5-HT concentration, but a large increase in the production of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), indicating an increase in the rate of synthesis of 5-HT, which is then broken down by MAO. No gross behavioural changes occur. Administration of the same dose of tryptophan to rats pretreated with tranylcypromine, a MAO inhibitor, produces a large rise in brain 5-HT. This dependence of the rate of 5-HT synthesis on brain tryptophan concentrations is now well documented.

The increase in 5-HT is associated with characteristic behavioural changes, one of which is hyperactivity which can be measured on activity meters (Fig. 1)[49]. Appearance of the hyperactivity syndrome is dependent on MAO inhibition, tryptophan administration and tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase activity (inhibition of either of these two enzymes with p-chlorophenylalanine and 4-bromo-3-hydroxybenzyloxyamine respectively, abolishes the hyperactivity (Fig. 1 and ref. 49)).

There is a positive correlation between the rate of accumulation of brain 5-HT and the rate of development of the hyperactivity, which suggests that the syndrome is produced by 5-HT[49]. Jacobs and colleagues have also demonstrated that the syndrome is the result of 5-HT stimulation[50,51]. Their studies[52] have shown that many of the behavioural aspects of the syndrome are present in the mesencephalic rat indicating that these behavioural changes are mediated by the B_1–B_6 group of neurones, which includes the pontine and medullary raphe nuclei and their synaptic contacts in the lower brainstem and spinal cord.

Our own recent biochemical studies[53] have strengthened the view that it is 5-HT and not a derivative that is responsible for the behavioural change, and we do not believe tryptamine is involved. The hyperactivity following tranylcypromine and tryptamine administration is inhibited by reserpine pretreatment[54], whereas that produced by tranylcypromine and L-tryptophan, far from being inhibited, has a more rapid onset following pretreatment with either reserpine or tetrabenazine, which block the storage or binding capacity of the synaptic vesicle[49].

The results of these experiments have been interpreted as follows (see also ref. 55). When tryptophan alone is given, the extra 5-HT synthesised is prevented from spilling-over into functional activity by intraneuronal binding and oxidative deamination. When MAO is inhibited the extra 5-HT accumulates at postsynaptic receptors, resulting in the hyperactivity syndrome. When intraneuronal binding is decreased by previous administration of reserpine or tetrabenazine and then MAO inhibited and tryptophan given, hyperactivity is enhanced because the "buffering" effect of vesicular binding against excess 5-HT production has been removed and more 5-HT "spills over" into functional activity.

This interpretation of the data has other implications. First, it suggests that the availability of tryptophan and the rate of 5-HT synthesis may not be very important in the fine regulation of the functional activity of 5-HT. Clearly the availability of tryptophan alters brain 5-HT metabolism but it seems unlikely that it is crucial in controlling the moment to moment functional activity of brain 5-HT. If synthesis were the only factor regulating the functional activity of 5-HT then it would follow that for each molecule of 5-HT released, a molecule would have to be synthesised and synthesis would therefore have to be critically and finely regulated from millisecond to millisecond to control synaptic events.

The second implication is that intraneuronal metabolism and binding of 5-HT are very important in controlling its functional activity. The third implication is that if a proportion of 5-HIAA is being produced intraneuronally from 5-HT which has not been functionally active then the measurement of 5-HIAA in the cerebrospinal fluid is likely to be of limited value as an index of the functional activity of 5-HT (see ref. 56 and later in the discussion of clinical implications).

Because 5-HT is stored in synaptic vesicles and decreasing this storage capacity alters the functional activity of 5-HT, compartmentation of 5-HT at the nerve ending might play an important role in regulating its functional activity and it has been suggested[49] that there is a prefunctional or reservoir pool, which nevertheless may be in equilibrium with the functional pool.

Unfortunately, at present the mechanisms involved in controlling the release of 5-HT are largely unknown so that fruitful discussion of this aspect is not possible.

To be able to investigate whether the drugs are acting on presynaptic mechanisms or postsynaptically the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) has been used. This compound causes a syndrome of hyperactivity qualitatively similar to that seen after tranylcypromine and tryptophan, but with a different time course[57]. It has therefore been used to bypass the effects of drugs on 5-HT synthesis, compartmentation or release and to investigate postsynaptic events.

Effects of lithium, diphenylhydantoin and chlorimipramine

Pretreatment of rats with one injection of either lithium[58] or diphenylhydantoin[41] causes the hyperactivity following tranylcypromine and L-tryptophan to begin earlier. There was no increase in 5-HT synthesis following one injection of either drug nor any change in response to 5-MeODMT indicating that postsynaptic responses were unaltered. Continued treatment with either of these two drugs caused an increase in 5-HT synthesis rate of 70% (lithium) and 100% (diphenylhydantoin).

It has been proposed that these two drugs may alter the compartmentation of 5-HT at nerve ending[58,59] thereby increasing the amount of 5-HT available for release and functional activity and that then leads to the increased rate of 5-HT synthesis. There is recent indirect evidence that diphenylhydantoin[60] (see also ref. 61) and lithium[62,63] increase brain 5-HT turnover in humans.

Inactivation of released monoamine by reuptake into the presynaptic nerve ending is well established[64,65]. We wished to see whether treatment with a known inhibitor of 5-HT reuptake would alter the hyperactivity syndrome. Pretreatment of rats with chlorimipramine before tranylcypromine and tryptophan caused the hyperactivity to begin earlier[41]. This may be because 5-HT cannot be inactivated by reuptake, and the concentration at the postsynaptic receptor site is therefore increased. One injection of chlorimipramine produced changes in the hyperactivity syndrome identical to those seen after one injection of diphenylhydantoin or lithium. But injection of chlorimipramine (25 mg kg^{-1}) twice daily for 2 d, with measurement of 5-HT synthesis on day 3 produced a 60% inhibition of 5-HT synthesis[41] in close agreement with a previous report[40], in contrast to the increased 5-HT synthesis seen after diphenylhydantoin or lithium. These findings suggest that diphenylhydantoin and lithium are not acting by inhibiting the reuptake of 5-HT.

Postsynaptic events

Finally we come to postsynaptic events as mechanisms for controlling 5-HT functional activity. Increasing the concentration of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the brain decreases the hyperactivity resulting from tranylcypromine and either tryptophan or 5-MeODMT administration[66].

Similarly, depletion of brain dopamine inhibited the hyperactivity from these two treatments[67]. Two interpretations can be made of this. Either a group of dopaminergic neurones lies between the 5-HT neurones responsible for initiating the hyperactivity response and those mechanisms responsible for its behavioural expression[67] or a group of dopaminergic neurones modulates the through-put from the activation of postsynaptic 5-HT receptors to the brain mechanisms responsible for the hyperactivity. It seems that this dopaminergic system may be involved in regulating the magnitude of the 5-HT behavioural response[68].

It had previously been shown that chlorpromazine inhibited the 5-HT induced hyperactivity without altering the rate of 5-HT accumulation[57]. It also inhibited the hyperactivity following 5-MeODMT. This suggested that it was acting at a postsynaptic site, probably by blocking 5-HT receptors[57]. Since there is good evidence that chlorpromazine blocks dopamine receptors[69,70] it now seems reasonable to suggest that it is inhibiting hyperactivity by interfering with the function of the dopaminergic mechanism, discussed above. Furthermore, recent studies (A. R. G. Heal, Boullin and D. G. G.-S., unpublished) have shown that other neuroleptic drugs, haloperidol, spiroperidol and α-flupenthixol (but not the clinically inactive isomer β-flupenthixol) also block 5-HT induced hyperactivity. All these drugs (except β-flupenthixol) also block the locomotor activity produced by a MAO inhibitor and L-dopa, strengthening the view that these drugs inhibit 5-HT responses by their action on dopaminergic neurones, though a direct effect to block 5-HT receptors has not been excluded.

Recently, we have found evidence that the sensitivity of the postsynaptic 5-HT receptor may be altered by electroconvulsive shock[71] While the short term changes of brain 5-HT metabolism seen in rats after a single electroconvulsive shock[71-73] cannot explain its therapeutic effect in humans, repeated electroconvulsive shock produces enhanced behavioural responses following 5-HT receptor stimulation. This may be because of a change in the sensitivity of the dopaminergic system involved in the 5-HT hyperactivity syndrome since postsynaptic dopaminergic responses are also enhanced by this treatment[71,74].

There is strong evidence that thyrotropin releasing hormone (TRH) over a restricted dose range, causes an enhancement of the hyperactivity that follows administration of tranylcypromine and L-tryptophan[75]. The effect was seen in hypophysectomised rats, and so could not have involved the pituitary–thyroid axis; nor did TRH alter the concentrations in the brain of 5-HT, dopamine or noradrenaline, or the rate of accumulation of 5-HT after administration of tranylcypromine and tryptophan. On the other hand, it did enhance hyperactivity after 5-MeODMT, which suggests a postsynaptic action on the response to 5-HT.

TRH has been shown not only to modulate several functions of dopaminergic systems[76-78] but to alter responses to several centrally acting drugs[75,79,81] and both in these and in the case of the hyperactivity syndrome, the administration of inhibitors of protein synthesis seems to act in a way that is generally "opposite" to that of TRH[80,81]. Since the synthesis of 5-HT itself was not inhibited by the drugs, it seems likely that this is another instance in which alterations in dopaminergic transmission influence the functional activity of 5-HT. Although it has yet to be demonstrated that TRH and the protein synthesis inhibitor cycloheximide act at the same site in the brain, both clinical and experimental evidence so far suggest that TRH may enhance[81-84], whereas cycloheximide inhibits, the release of dopamine in rat brain[81]. TRH is widespread in the brain[85-86] and unpublished observations (quoted in ref. 87) suggest that it is present in nerve endings along with a specific enzyme for degrading it, all of which is consistent with a modulatory role.

If polypeptides or proteins do mediate or modulate the actions of brain monoamines, then malfunction of such compounds might be confused with a primary abnormality in monoamine function. We found that although treatment with cycloheximide prevented the appearance of the hyperactivity syndrome after the usual doses of 5-MeODMT, increasing the dose of 5-MeODMT overcame this block[80]. It is just possible that in some psychiatric diseases attributed to primary abnormalities in monoaminergic function and which appear to respond to measures increasing or inhibiting monoaminergic activity, the abnormality lies with polypeptide or protein modulators.

In summary it is proposed that the functional activity of brain 5-HT is controlled predominantly by the following factors: (1) The availability of tryptophan to tryptophan 5-hydroxylase; (2) the regulation of the amount of 5-HT available for release by intraneuronal MAO activity and compartmentation such as vesicular storage mechanisms; (3) reuptake of 5-HT from the synaptic cleft; (4) sensitivity of the postsynaptic receptor; (5) regulation of the total postsynaptic neuronal responses through the influence of neuronal systems utilising other neurotransmitters such as dopamine. These processes are outlined in the hypothetical scheme presented in Fig. 2 with indications of the points at which various drugs are thought to act.

Clinical implications

The ethical problems posed by experimental psychopharmacology in humans are daunting. The introduction of really innovative drug therapy in psychiatric disease involves bridging the gap between experimental neuropharmacology and the manipulation of animal behaviour (which is fairly crude) and the manipulation of the neuropharmacology of the human brain to bring about subtle changes in the mind (which is likely to be a much finer process). What conclusions can be drawn from these studies of 5-HT function in rat brain, as mirrored by manipulation of the hyperactivity syndrome, which might help to bridge this gap?

Tryptophan alone produces an increase in the rate of 5-HT synthesis in both the rat brain and in the human brain (if 5-HIAA levels in cerebrospinal fluid can be relied on[56]), but neither hyperactivity in the rat nor, probably, relief of depression in the human results. A simple lack of brain 5-HT is therefore probably not responsible for the depressive state. Yet tryptophan plus a monoamine oxidase inhibitor produces hyperactivity in the rat and is an effective therapy for depres-

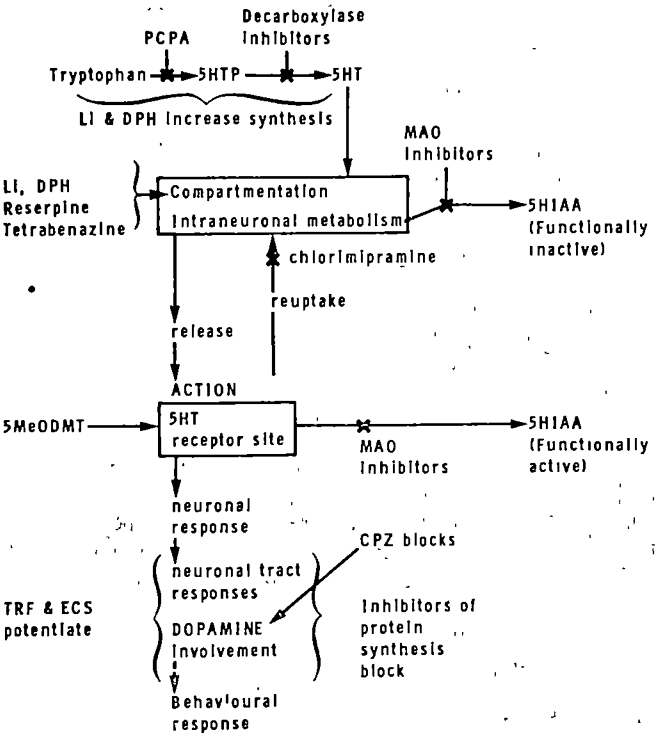

Fig. 2 Some postulated processes regulating 5-HT function and effects of drugs upon them. PCPA, *p*-chlorophenylalanine; 5-HTP, 5-hydroxytryptophan; 5-HT, 5-hydroxytryptamine; MAO, monoamine oxidase; 5-HIAA, 5-hydroxyindoleacetic acid; Li, lithium; DPH, diphenylhydantoin; 5-MeODMT, 5-methoxy-*N,N*-dimethyltryptamine; TRF, thyrotropin releasing factor; ECS, electroconvulsive shock; CPZ, chlorpromazine

sion[88]. In humans only relatively small doses of tryptophan can be used and MAO cannot be fully inhibited, so the problem is to find other mechanisms for increasing 5-HT function if indeed this is the mechanism by which tryptophan and a MAO inhibitor produce their therapeutic effect. For instance lithium or diphenylhydantoin might be used to render more 5-HT available for functional activity in combination with tryptophan and/or MAO inhibitors. Reserpine or tetrabenazine together with MAO inhibitors and tryptophan if carefully used may prove effective in retarded depression resistant to other forms of therapy[89]. Chlorimipramine, which blocks 5-HT reuptake, may in combination with MAO inhibitors and/or tryptophan also increase 5-HT function fairly selectively and might be used to chart those depressive states amenable to increasing 5-HT function. One might ask, why not just give a 5-HT agonist? 5-MeODMT and *N,N*-dimethyltryptamine while producing the hyperactivity syndrome are potent hallucinogens[90] but as far as we are aware small sub-hallucinogenic doses have not been tried in depressive states and perhaps the risk is too great. The possibility of more selective 5-HT agonists remains, however.

The similarity between the actions of lithium and diphenylhydantoin on brain 5-HT function prompts us to suggest the trial of diphenylhydantoin in the prevention of manic-depressive diseases. The adverse effects of diphenylhydantoin are well known through its long use in the treatment of epilepsy and they are certainly no worse than those already encountered with lithium. It should also be considered whether when a patient on "prophylactic" lithium for manic-depressive disease goes into a depressive state, MAO inhibitors given in conjunction with lithium might not be the best therapy.

For many years the presence of an endogenous psychotogen in schizophrenia has been sought, sometimes on the assumption that such an endogenous psychotogen could be produced by an abnormal metabolic pathway or by an abnormally functioning normal metabolic pathway. Because 5-MeODMT, itself an hallucinogen (and which has been invoked as an endogenous psychotogen in schizophrenia[91,92]), causes the same hyperactivity syndrome as is produced by raised 5-HT levels, we would suggest that abnormal functioning or increased activity of normal transmitters in their normal neuroanatomical pathways could equally be responsible for such changes. The worsening of the schizophrenic state reported after the administration of tryptophan and a MAO inhibitor[93] could be interpreted in this light. Since we have seen that postsynaptic mechanisms can change the behavioural response to brain 5-HT, schizophrenia could involve a change in the sensitivity of neuronal responses to "normal" stimuli.

The apparent interplay of dopamine and 5-HT in the production of the hyperactivity syndrome makes one wonder whether in psychiatric states some kind of imbalance occurs between neuronal systems containing 5-HT and dopamine, just as imbalances between cholinergic and dopaminergic systems seem to be involved in Parkinsonism[94]. These speculations are testable clinically using tryptophan, 5-HTP, and L-dopa, with or without (in the latter two cases) peripheral aromatic amino acid decarboxylase inhibitors.

The finding that chlorpromazine and some other neuroleptics, which it is generally agreed, block dopaminergic receptors in the brain, can probably by this action also block the sequences necessary for the production of the behavioural response to 5-HT, shows that these drugs may not be acting at a primary level, but rather at a secondary level in the therapy of schizophrenic illness. This has already been pointed out[8]. Nevertheless and in the loosest sense, perhaps the phenothiazines are capable of blocking the dopamine gates (and there may be several of them) through which several more primary stimuli may pass. There is no doubt that we need drugs which are not only pharmacologically more specific as agonist–antagonists but also show neuroanatomical specificity of function.

Finally, there is the controversy about the usefulness of 5-HIAA concentrations in the cerebrospinal fluid as an index of the functional activity of 5-HT in humans. This has been dealt with in a comprehensive review[56]. In animal studies 5-HIAA in the cerebrospinal fluid (CSF) does seem to reflect central 5-HT concentrations[95,96]. Furthermore, drugs affecting 5-HT turnover alter 5-HIAA in the lumbar CSF of human subjects[97-99]. Against this it has been shown that 5-HIAA injected into the cisterna magna of the cat does not change lumbar CSF 5-HIAA[99] although this is disputed[100]. Furthermore, patients with a spinal block at the thoracic level do not have decreased 5-HIAA[101,102]. Another study, however, showed substantially decreased CSF 5-HIAA in patients with an incomplete block[103].

Garelis and coworkers[56] argue convincingly that CSF 5-HIAA reflects central metabolic changes of 5-HT. If our earlier conclusions regarding the intraneuronal organisation of 5-HT synthesis, compartmentation and metabolism are correct, however, 5-HIAA comes not only from 5-HT that has been functionally active but from 5-HT synthesised in "excess of needs" which has been metabolised by monoamine oxidase intraneuronally. This means that CSF 5-HIAA concentrations include both of these "pools" of 5-HIAA and does not necessarily reflect the metabolism of 5-HT which has been released and has been functionally active.

Until we can measure that proportion of 5-HIAA resulting from the metabolism of functionally active 5-HT (and at present we have no idea under normal situations what this proportion might be) it is premature to conclude that changes in CSF 5-HIAA levels[56], or rates of rise of 5-HIAA in CSF after probenecid treatment with or without tryptophan loading[104], really indicate the functional activity of 5-HT in the brain. This is not to say that if consistent changes in CSF 5-HIAA are found to occur in certain affective disorders, the knowledge may not be empirically useful; but only to point out the hazards of interpreting the data in terms of the functional activity of 5-HT and its primary aetiological involvement in psychiatric disease.

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The directional dependence of the primary cosmic rays of energies 10^{11} - 10^{12} eV

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The apparent sidereal variation in the intensity of muons generated by cosmic rays with energies greater than 10^{11} eV showed a marked change in phase at Northern Hemisphere stations at the time of the recent reversal in sign of the general solar magnetic field. No corresponding change was observed at rather lower energies at Hobart, Tasmania. By adopting a particular model of the large scale interplanetary field, it is possible to account for this behaviour of the sidereal variation in terms of a primary cosmic ray anisotropy which is related to the local direction of the galactic magnetic field.

SYSTEMATIC long term studies of the variations in intensity of muons deep underground have been carried out by a number of investigators to establish the existence of a variation over the sidereal day. The depths at which the observations relevant to this paper have been carried out lie in the range 30–80 m water equivalent (m.w.e.). At a depth of 60 m.w.e. most of the muons derive from primaries with an energy per nucleon of 10^{11}–10^{12} eV.

An experimental result that has become rather well established over the years is that in the Southern Hemisphere at Hobart (depth 32 m.w.e.) the sidereal variation has an amplitude of ~0.030% with a time of maximum close to 0600 LST whereas stations in the Northern Hemisphere, Budapest (40

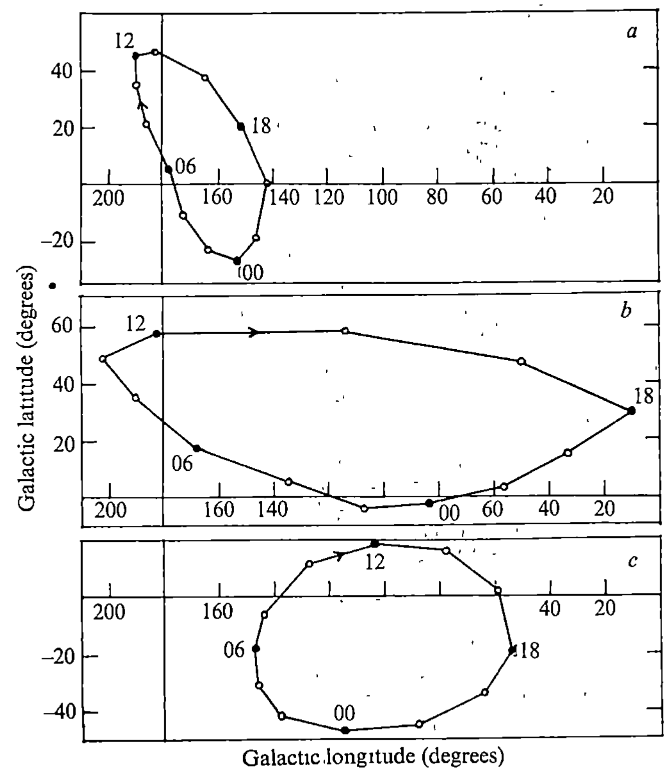


Fig. 1 Galactic scan directions for the vertical direction in London of the epoch 1 interplanetary field configuration. *a*, Average for October–February; *b*, for March–May; *c*, for June–September.

m.w.e.), London (60 m.w.e.), Socorro (80 m.w.e.) and Torino (80 m.w.e.), showed a rather smaller variation with a time of maximum displaced by almost 12 h from that in the Southern Hemisphere. Sekido[1], using a Cerenkov telescope to select high energy muons at ground level which are the progeny of primaries in the same primary energy band as those generating the underground muons, has obtained results which are in substantial agreement with the underground measurements.

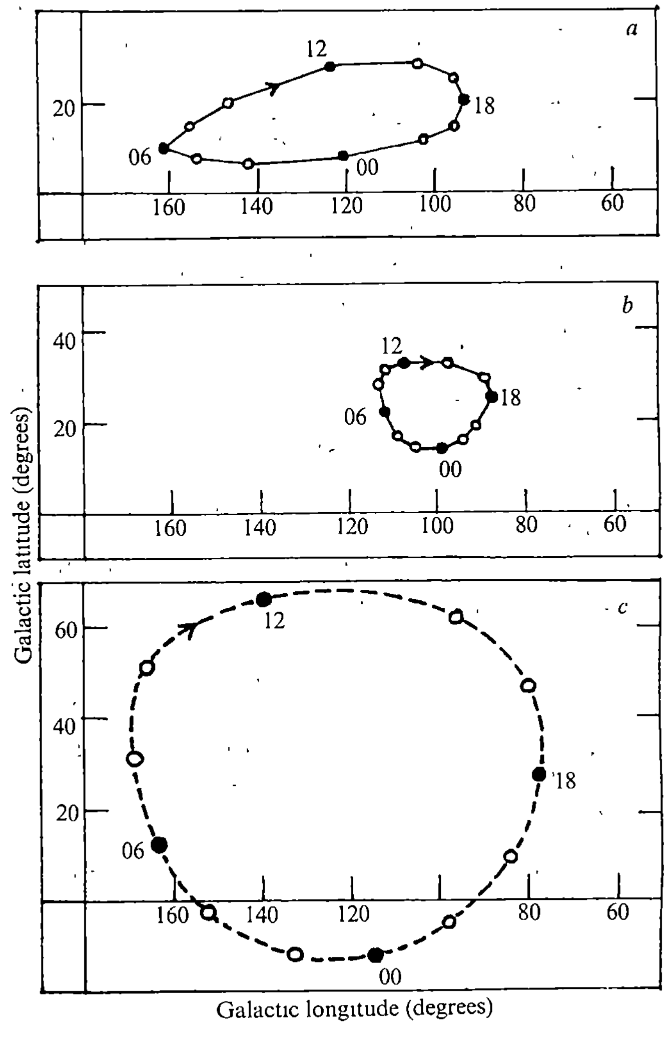
Jacklyn[2] and Sekido[1] have interpreted these experimental results in terms of a 'two-way' anisotropy of the primary cosmic rays in space. That is to say the intensity distribution has two maxima located on the celestial sphere in such a way as to be compatible with the observed times of maximum in the Northern and Southern Hemispheres. This assumes that the directions on the celestial sphere that are scanned by the underground cosmic ray detectors are not significantly affected by the interplanetary magnetic field. In other words the directions in space from which the primary cosmic rays arrive were taken to be those corresponding to the purely geometrical projection of the cone of acceptance of the telescopes on to the celestial sphere after allowing for the (small) deflection in the geomagnetic field. In fact we shall show that the presence of the interplanetary field changes very substantially the scanning directions of underground telescopes at the depths we are considering.

Speller *et al.*[3] and Barnden[4] have examined the effects of an interplanetary magnetic field model consisting of sectors in the meridian plane of alternate polarity on the trajectories of cosmic rays in the range of magnetic rigidities appropriate to muon detectors at 60 m.w.e. In particular Speller *et al.*[3] showed that the intensity variation over the sidereal day observed in the Northern Hemisphere could be accounted for by the motion of the solar system relative to the average rotation frame of the Galaxy in our neighbourhood. The cosmic rays are assumed to rotate with the Galaxy and the relative motion of an observer in the solar system then leads to a sidereal variation in close agreement with the observed amplitude and phase. The variation observed at Hobart could not, of course, be explained in this way since the time of maximum differs by 12 h from that expected for the solar motion effect, and in addition the amplitude is too large.

During the period after 1970 the sidereal variation observed at stations in the Northern Hemisphere changed markedly from that found in the earlier years. Data from the vertical recorders at the Holborn laboratory in London show that the sidereal wave has shifted in phase by ~180 ° so that the time of maximum is now ~0600 and has been for the past few years. Similar changes in phase have been observed at Torino by Cini-Castagnoli (personal communication) and by Swinson[5] for his Socorro station. This change in phase[6] seems to have taken place at the same time as the reversal of the large scale solar photospheric fields[7]. Neither the solar field nor the sidereal variation itself changed abruptly and, as far as can be seen, the change was spread over several years. For convenience, however, we assume here that the change took place at the end of 1969 and we shall refer to the period before the changeover as epoch 1 and the period from 1970 on, following the changeover, as epoch 2.

The change in phase of the sidereal wave is apparently confined to the Northern Hemisphere stations and has not occurred at Hobart[9]. As concluded by Cini-Castagnoli *et al.*[6] it is very likely connected with a change in the interplanetary field which is in turn related to the magnitude and sign of the photospheric fields. We have therefore been led to investigate

Fig. 2 Galactic scan directions for the vertical direction in London of the epoch 2 interplanetary field configuration. *a*, Average for April–July; *b*, for remaining months; *c*, scan for infinite energy particles.



the effects on cosmic ray trajectories of a rather different interplanetary field model from that used by Speller *et al.*[3] and by Barnden[4].

The interplanetary field model

It is clear that the changes in the large scale photospheric fields observed by Howard[7] must produce corresponding changes in the interplanetary magnetic field and evidence for such changes based on *in situ* spacecraft magnetometer measurements has been given by Rosenberg[9] and by Hedgecock[10]. There is no doubt that the presence of the interplanetary magnetic field affects the trajectories of primary cosmic rays of magnetic rigidity $\leqslant 10^{12}$ V which generate the bulk of the muons monitored at depths $<$ 80 m.w.e. and it is to be expected, therefore, that a substantial change in the field will in some measure influence the character of the sidereal variation.

As stated above the effect on such primaries of an interplanetary field with a 2- or 4-sector meridional structure has been investigated by Speller *et al.*[3] and by Barnden[4] and these authors have shown that this type of field does not scatter the relevant cosmic rays sufficiently to prevent the observation of a galactic anisotropy at the Earth although it does reduce the measured amplitude. It now seems possible, however, that the interplanetary magnetic field is not of this sectored form, but, instead, derives from a large scale solar dipole in which the field north of the solar equatorial field is predominantly of one sign while that south of the equatorial plane has the opposite sign[11,12]. The two-sector structure of the interplanetary field, which is the most frequently observed configuration, is then attributable to the tilt angle between the axis of the solar dipole and the Sun's rotational axis.

A field of this configuration has a much more serious perturbing effect on cosmic ray trajectories than one which is meridionally sectored, where the alternating sign of the field is to some extent self-cancelling so far as the particle deflections are concerned. Consequently there is good reason to suppose that the reversal of such a stretched dipole field would produce a significant change in the form of the cosmic ray anisotropy observed at the Earth.

In determining the directions on the celestial sphere scanned by an underground monitor in the course of the day it is necessary first to compute the trajectories of representative cosmic ray primaries which reach the Earth at the appropriate latitude and longitude for a variety of times of day throughout the year and then combine these with the response functions of the detector[13,14]. The interplanetary field model we have used is a simple idealised one of the form

$$B_r = B_0 \left(\frac{a}{r}\right)^2$$

$$B_\theta = 0$$

$$B_\varphi = B_0 \left(\frac{a}{r}\right) \sin\theta$$

where θ is the heliographic colatitude and B_0 is the radial component of the field at the Earth's orbit ($r = a$). Although, as stated above, the dipole axis must be inclined to the Sun's axis of rotation we do not know what this angle is, and for the purpose of the present calculation it has been taken to be zero. We have also assumed that to a sufficiently good approximation the radial and tangential components of the field at 1 AU can be taken as equal and written as $B_0 = 2.5$ nT. B_0 is positive in sign (the field is directed away from the Sun) everywhere north of the solar equatorial plane and negative everywhere to the south of it for epoch 2. The field in epoch 1 has been taken to be the exact reverse. These field directions correspond in sign to those measured in the photosphere. The trajectory integrations were terminated at a heliocentric distance of 30 AU, under the assumption that the field is ineffective at greater distances but extending the field model and the integration process to 100 AU does not significantly change the asymptotic directions of the trajectories.

Using these two field models, average viewing directions weighted according to the response function for a depth of 60 m.w.e. at London have been computed at 2-h intervals throughout the sidereal day for each month of the year for the two epochs. Certain months have very similar directional scans and to reduce the number of diagrams these have been combined together. Figures 1 and 2 show, in galatic coordinates, the effective 'direction of look' of the vertical underground telescopes as a function of sidereal time.

As Figs 1 and 2 show, reversal of a stretched dipolar field of the kind used in the trajectory computation has a significant effect on the directions scanned by the Holborn muon monitor in the course of the sidereal day. The corresponding purely geometric scan for infinite energy particles or zero interplanetary magnetic field is shown in Fig. 2 for comparison and it will be clearly evident that if the interplanetary field is of the form assumed it must be taken into account in interpreting the sidereal variation in terms of an anisotropic distribution of cosmic rays in space.

Cosmic ray intensity contours

The radius of curvature of the trajectory of a cosmic ray with magnetic rigidity 3×10^{11} V in the galatic magnetic field of ~ 0.3 nT is $\sim 3 \times 10^{14}$ cm which is very small compared with the dimensions of a spiral arm. It seems highly improbable that the transport mean free path in interstellar space can be much less than 1 pc, and consequently the motion of the primary cosmic rays we are concerned with can be considered to be nearly adiabatic in the galactic field. In these circumstances it is plausible to regard the cosmic ray directional distribution as essentially a pitch angle distribution having symmetry about the local direction of the magnetic field whatever that may be. If this picture is valid we would then expect the angular distribution of the cosmic rays to be symmetrical about the two points on the celestial sphere where it is intersected by the local field vector.

With this assumption of symmetry and the directional scans of Figs 1 and 2, the measured intensity variation over the sidereal day can be used to construct cosmic ray intensity contours for galactic longitudes in the range 0–200°. In doing so, it is necessary to correct the measured sidereal variation for two separate solar effects.

The first of these is the solar motion effect referred to above which can be calculated from the known solar velocity, the exponent of the cosmic ray spectrum in the relevant energy region and the directional scan diagrams. The second correction arises from the solar diurnal temperature wave in the terrestrial atmosphere which generates a spurious sidereal time variation of the muon intensity because of its seasonal dependence. The variation in the muon intensity underground which is generated in this way is a combination of pion and muon decay effects. The former leads to a positive temperature coefficient whereas the latter has the opposite sign. We have not attempted to calculate this correction from the (uncertain) atmospheric temperature variation but have instead made use of the spurious sidereal variation measured by inclined telescopes situated at the same depth in Holborn with their directions of maximum sensitivity directed along the Earth's axis of rotation. The 'sidereal variation' observed in this way arises entirely from atmospheric temperature variation, since such telescopes do not see any genuine variation from a primary cosmic ray anisotropy.

The intensity contours derived in this way are shown in Fig. 3, and Fig. 4 illustrates the degree of fit attained between the experimental data and these contours. In the latter diagram 2-h departures from the mean intensity over the sidereal day at Holborn averaged for the two epochs and corrected for the

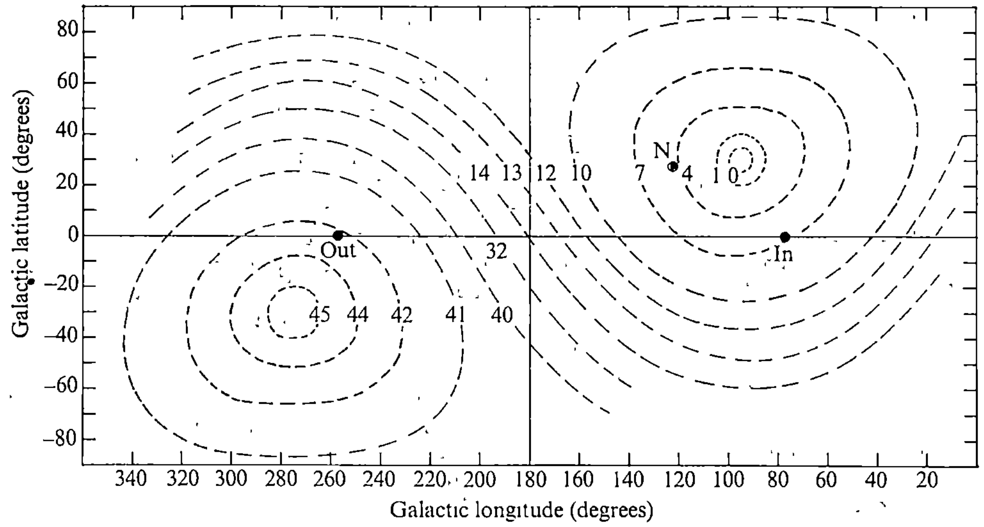


Fig. 3 Intensity contours in galactic coordinates inferred from the sidereal variation observed in London and Hobart. They are in units of 7.5 × 10^{-5}. 'In' and 'Out' correspond to the local spiral arm in and out directions. N is the direction corresponding to the Earth's North Pole.

two solar effects described above have been plotted together with the variation to be expected on the basis of Fig. 3.

To extend the contours derived from the London data to cover the complete range of galactic longitudes we have made use of observational data from Hobart together with galactic scan diagrams for that station, similar to those in Figs 1 and 2. It turns out that while the Hobart scans extend the range of longitudes covered beyond 200°, they also show considerable overlap with those for London, which permits a satisfactory patching together of the data. Figure 5 illustrates the degree of fit between the Hobart measured first harmonics and those deduced from the intensity contours of Fig. 3 for the two epochs. It is of particular interest to note that reversal of the interplanetary magnetic field does not reverse the phase of the sidereal variation in Hobart as it does in London.

Conclusions

In summary we believe that the observational data on the sidereal variation of cosmic rays in the energy range 10^{11}–10^{12} eV in both the Northern and Southern Hemispheres, including the phase reversal in the former, are all compatible with the intensity distribution shown in Fig. 3, provided that the field configuration in the interplanetary field model correctly describes the magnetic conditions in interplanetary space. The axis of symmetry of the cosmic ray distribution, which on this model should correspond to the local direction of the interstellar magnetic field, lies at ~25° to the Earth's axis of rotation. It is fortunate that the angle is not smaller, since the possibility of observing an anisotropy of the kind discussed here diminishes rapidly as this angle tends to zero, even for high energy particles where the effect of the interplanetary field is insignificant.

It has often been argued that the presence of the interplanetary magnetic field makes it impossible to observe any anisotropy for primary cosmic rays in this range, and that because of this, it is necessary to make such measurements at much higher energies, where the effect of the field on the particle trajectories is negligible. Our result is somewhat ironic in that the presence of the interplanetary magnetic field apparently results in a more favourable directional scan for the lower energy particles than for high energies because of the small angle between the Earth's axis and the axis of symmetry of the cosmic ray distribution.

Fig. 4. Corrected average 2-h departures of the cosmic ray intensity from the mean in London for epochs 1 (*a*) and 2 (*b*) together with the variation (dotted curves) expected from Fig. 3.

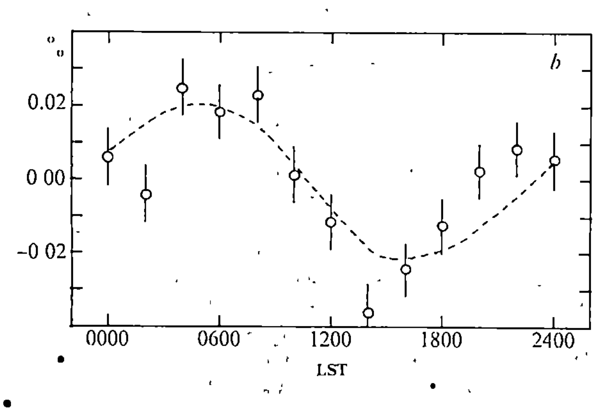
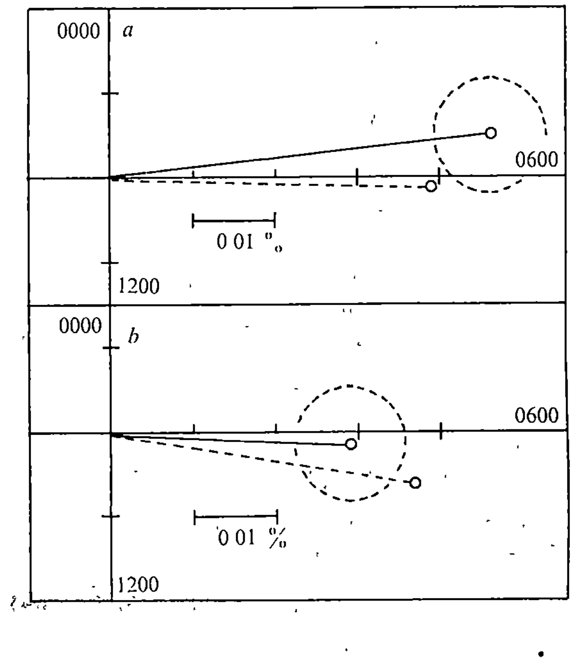


Fig. 5 Harmonic dials showing the 24-h sidereal waves to be expected from Fig. 3 (– – –) together with those measured in Hobart (——) for epochs 1 (*a*) and 2 (*b*).



The question as to whether an apparent sidereal anisotropy can be simulated by purely local solar effects (see for example Swinson[16]) has often been discussed in the past and in this connection it should be borne in mind that the axis of symmetry of the distribution in Fig. 3 lies along the normal to the ecliptic, so that any preferential solar induced streaming of the cosmic rays perpendicular to the ecliptic plane could in principle produce an angular distribution similar to that described here. It does not, however, seem possible to reconcile other established characteristics of the sidereal variation with such a solar effect. In particular we are not aware of any solar process which is capable of explaining the change in phase of the siderial variation observed at Northern Hemisphere stations in 1969–70 together with the absence of any corresponding change at Hobart. In the absence of any satisfactory explanation based on solar processes alone it is our opinion that the angular distribution shown in Fig. 3 is most readily interpreted in terms of a galactic magnetic field pitch angle distribution.

Because of the low statistical accuracy of the measured sidereal variation, the uncertainty of the corrections applied, the relative insensitivity of the observational method which is a consequence of the small angle between the axis of symmetry of the intensity distribution and the Earth's rotational axis and, above all, the uncertainty in the interplanetary field model, the contours of Fig. 3 cannot be more than a very rough approximation to the truth.

Accepting, however, that within rather wide limits of uncertainty the intensity distribution is valid, a plausible explanation for such a distribution can be given. The intensity minimum of Fig. 3 is taken to correspond to the galactic magnetic field direction leading inwards towards the centre of the Galaxy, a not unreasonable assumption since it lies closest to the 'spiral in' direction of the local arm. If, then, cosmic rays of energy $> 3 \times 10^{11}$ eV are predominantly of extragalactic origin, this pitch angle distribution could be the result of the extra amount of galactic material traversed by particles whose trajectories are such that they arrive at the Earth after reflection at some point in the spiral arm closer to the galactic centre than the Solar System and where the magnetic field is stronger than it is in the neighbourhood of the Sun. An additional path length corresponding to the traversal of ~ 0.3 g cm^{-2} of interstellar matter would suffice to explain the observed departure from isotropy.

This work was carried out with the aid of an SRC grant and we are indebted to Dr C. J. Hatton for drawing our attention to an error in the interplanetary field model used in earlier calculations.

Received December 4, 1975; accepted February 19, 1976.

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Genomic transcriptional activity and the structure of chromatin

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Nucleic acid hybridisation has shown that micrococcal nuclease-derived chromatin subunits from the cells of Xenopus laevis *contain fragments of ribosomal 28S, 18S and 5S RNAs within the population of 200 base-pair pieces of DNA. Subunits from cultured embryonic cells actively transcribing ribosomal RNA contain only 70–74% of the cistrons present in undigested wild-type DNA, while subunits from adult erythrocytes not active in RNA transcription contain close to 90% of the ribosomal cistrons in native chromatin.*

CHROMATIN can be cleaved, either by endogenous nucleases[1] or by exogenously supplied micrococcal nuclease[2,3-7] into fragments containing discrete size classes of protected double-stranded DNA. Noll[2] has reported that about 85% of rat liver chromatin is susceptible to such digestion giving rise to a basic "chromatin subunit" with a sedimentation vale of 11.2S and containing about 205 base pairs of DNA associated with nucleoproteins. Kornberg[4] has proposed a model for chromatin structure based on such DNA subunits being associated on the outside of an octamer of histones[8,9] with the composition f2a1$_2$ f3$_2$ f2a2$_2$ f2b$_2$. Van Holde[10] has proposed a similar chromatin model. Histone f1 is not present in these subunits[3-7]. Electron microscopic[11-14] and neutron diffraction studies[15] have strongly supported such a beads-on-a-string model for chromatin. Little is known, however, about the functional significance of such a chromatin substructure. It is thus interesting to ask whether the areas of chromatin that are more accessible to various chemical probes, such as nucleases[16-21], are also the areas more active in genetic transcription. And, conversely, to query whether those areas of chromatin protected from ready nuclease digestion represent less active, or perhaps, repressed parts of the genome[22]. We present here results of nucleic acid hybridisation experiments in which purified ribosomal RNAs (rRNAs) have been annealed to the DNA from chromatin subunits prepared by micrococcal nuclease digestion of isolated nuclei. Nuclei were obtained from tissue culture cells actively transcribing the ribosomal genes and from mature erythrocytes, which are not active in transcription. By comparing the susceptibility to nuclease digestion of DNA in both transcriptionally active and inactive states we hoped to obtain information concerning the structure and function of native chromatin.

Isolation of chromatin DNA fragments

Nuclei isolated from embryonic *Xenopus laevis* tissue culture cells and treated with micrococcal nuclease (EC 3.1.4.7) for 4 min at 37 °C release, when lysed, pieces of chromatin that are integral multiples of a discrete particle size, the monomer

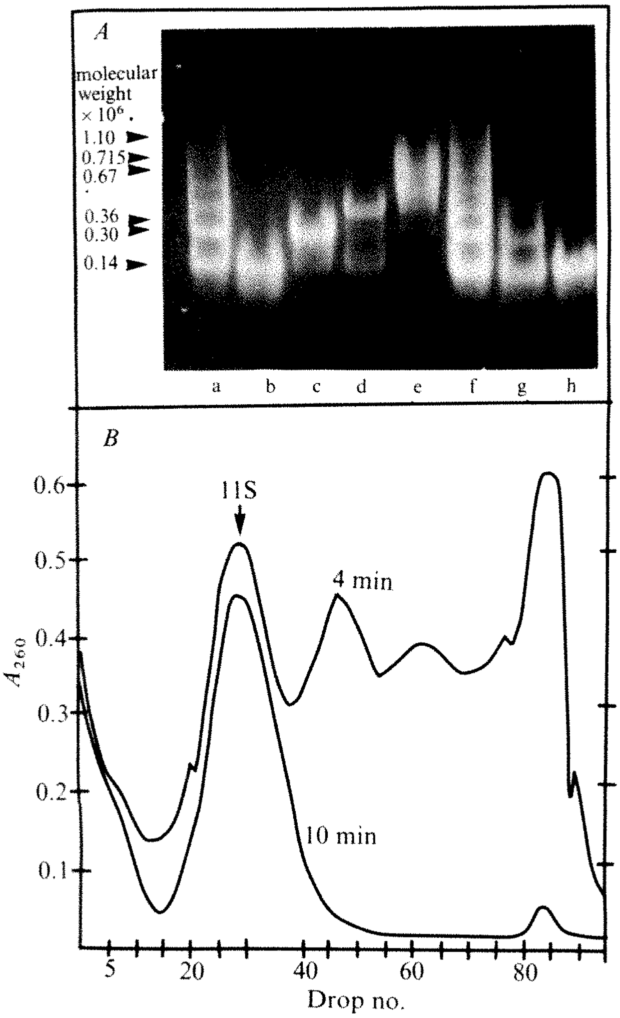

Fig. 1 *A*, Patterns obtained when DNA fragments were separated by electrophoresis on a 1.4% Agarose slab gel, stained with ethidium bromide and photographed under ultraviolet light using the methods of Sharp *et al.*[41]. The electrophoresis buffer contained 40 mM Tris base (*p*H 7.8), 5 mM sodium acetate and 1 mM EDTA. Electrophoresis towards the anode was for 2 h at room temperature on a Raven slab gel apparatus run at a constant 35 mA. Slot a: 15 μg of DNA fragments isolated from tissue culture cell nuclei that had been incubated with micrococcal nuclease (500 U ml^{-1}) for 4 min at 37 °C; Slots b–e: DNA fragments isolated from tissue culture cell monomer, dimer, trimer and tetramer (and larger) chromatin subunits separated by centrifugation on a 10–30% sucrose gradient run for 20 h at 30,000 r.p.m. in a Beckman ultracentrifuge using a SW 50.1 head; slots b–e contained, respectively, 10 μg, 10 μg, 5 μg, and 10 μg of DNA each. Slots f–h: sequence showing DNA fragments from timed nuclease digestions of tissue culture cell chromatin; slot f: 4-min digest of nuclei (30 μg DNA); slot g: 8-min digest (10 μg of DNA); slot h: 10-min digest (10 μg of DNA). *B*, Absorbance profile (at 260 nm) of chromatin subunits from actively growing tissue culture cells separated on a 10–30% sucrose gradient as described in (*A*). The sucrose solutions contained 0.2 mM EDTA and 2.85 mM phenylmethylsulphonylfluoride (PMSF). After centrifugation the gradients were pumped through a Uvicord II recording UV analyser and separated into fractions by drops. The upper curve gives the profile from a 4-min nuclease digest (at 37 °C) and the bottom curve the profile of a 10-min digest of tissue culture cell chromatin. Catalase, as a convenient marker for the 11S region, was run on a separate gradient. For preparation and micrococcal nuclease digestion of nuclei, exponentially growing tissue culture cells were collected by standard procedures[42] and washed in homogenisation solution (10 mM Tris-HCl, *p*H 7.8, 1 mM $MgCl_2$, 0.05 M $NaHSO_3$, 0.15 M sucrose) at 4 °C. The cells were then centrifuged at 250*g* for 10 min and the pellet was resuspended in hypotonic homogenisation solution (above buffer without sucrose) and swollen for 10 min at 4 °C. After further pelleting the swollen cells were resuspended in complete homogenisation solution containing 0.5% Triton X-100 and pipetted vigorously. After washing and pelleting the isolated nuclei were resuspended in homogenisation medium (without Triton X-100) containing 1 mM $CaCl_2$. Micrococcal nuclease was added to the solution (with concentrations of nuclei of up to 5×10⁸ per ml) to give a final nuclease concentration of 300–500 U ml^{-1} depending on the experiment. The nuclei were nuclease digested at 37 °C for the times indicated and then pelleted at 6,000*g* for 10 min. Erythrocyte nuclei were isolated by the procedures of Destree[43]. Chromatin particles were freed from the pelleted nuclei by homogenisation in the complete homogenisation solution containing 0.2 mM EDTA and 2.85 mM PMSF at *p*H 8.0 and 4 °C. Nuclear membranes and insoluble materials were removed by centrifugation. The chromatin-containing supernatant was then concentrated and dialysed against the appropriate buffer solutions for a particular experiment. Protein determinations were either by the method of Lowry[44] or by the use of the E_{280}/E_{260} ratio using appropriate standards. To isolate DNA, whole chromatin or chromatin subunits derived by nuclease digestion were dialysed against SSC (0.15 M NaCl, 0.015 M trisodium citrate, *p*H 7.8) before extraction of DNA by the technique of Brown and Weber[27,28].

subunit. The chromatin subunits have a sedimentation value of about 11S when run on a sucrose gradient (Fig. 1). The double-stranded DNA isolated from these monomers has a molecular weight of about 1.4×10⁵ as judged by its comigration during electrophoresis with marker viral SV40 DNA endonuclease restriction pieces of known molecular weight[23,24]. In Fig. 1*A*, columns a and f show the Agarose gel electrophoretic pattern of DNA fragments isolated from nuclei digested with nuclease for 4 min at 37 °C. On the left of the figure are the molecular weights of marker SV40 DNA pieces electrophoresed on the same slab gel. In the 4-min digest there are at least six DNA bands visible under ultraviolet light in this slab gel stained with ethidium bromide. These bands are, as shown by the molecular weight markers, integral multiples of the approximately 200 base pairs present in the monomer DNA. As columns g and h show, with increasing times of nuclease digestion, the larger multimeric DNA bands disappear from the profile so that by 10 min of incubation (column h) 70–75% of the bulk DNA in these tissue culture cells has been converted to the 140,000-dalton monomer DNA size. Such 10-min digests of culture cell chromatin were thus routinely used as a source of monomer DNA for most of the hybridisation studies reported here.

In all of these DNA preparations from nuclease-digested chromatin a slight smear of DNA fragments, with a few weak but distinct minor bands, electrophoresed faster than the major monomer DNA band. These bands do not show up very well on this photograph but were always a very minor fraction of the total DNA present in any preparation.

When the chromatin fragments from a 4-min digest of culture cell nuclei were centrifuged on a 10–30% sucrose gradient the absorbance profile of the gradient shown in Fig. 1*B* was obtained. As is evident, it is possible to separate the oligomeric chromatin fragments of the digest according to increasing size and weight on such gradients. The monomeric subunit migrates with a sedimentation velocity of about 11S as determined by using a catalase marker. Figure 1*B* also shows the absorbance profile of chromatin from a 10-min digest confirming that by this length of nuclease treatment virtually all the remaining DNA of the culture cells is of the monomer DNA size class. When the DNA from each of the gradient peaks from a 4-min chromatin digest are extracted and electrophoresed on an

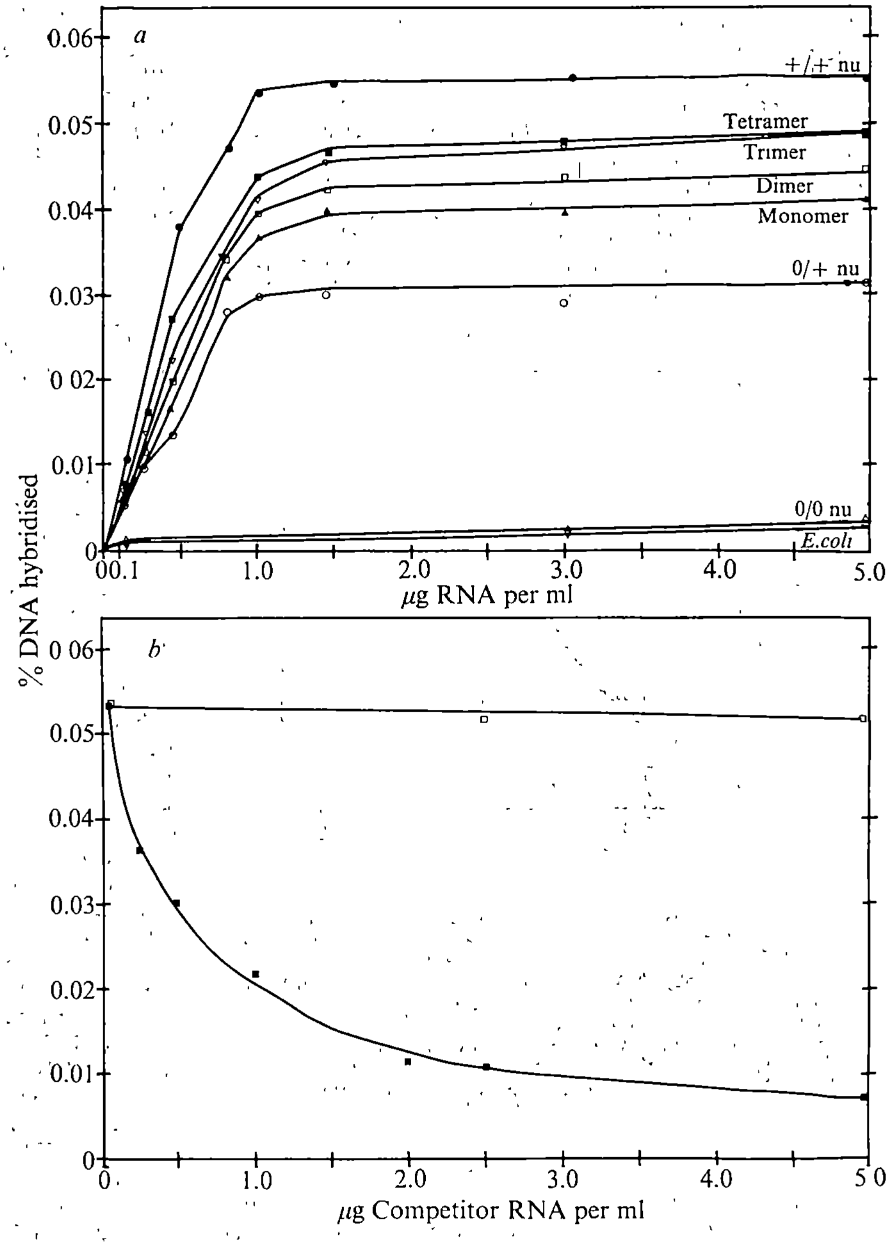

Fig. 2 *a*, Saturation profiles for hybridisations obtained from one experiment in which all DNA-containing filters were annealed in identical conditions with increasing concentrations of the same batch of purified ^{32}P-18S+28S RNA. The specific activity of the RNA was 728,000 c.p.m. per µg RNA. Monomer, dimer, trimer and tetramer (and larger) DNAs were isolated from logarithmically growing tissue culture cells as described in the legend to Fig. 1. Embryos of +/+ nu, 0/+ nu and 0/0 nu genotypes were obtained by mating two heterozygous anucleolate mutant frogs by standard techniques[45]; their DNAs were isolated as described. *E. coli* DNA was from Sigma. Curve codes as labelled. Each point is the average of the counts on two separate filters. Counts on filters were corrected for non-specific binding to blank filters (about 30 c.p.m.) and for loss of DNA from filters during the hybridisation. Each 6-mm filter initially contained 5–10 µg of immobilised DNA and during the annealing reaction no more than 10% of the DNA was lost from the filters. Loss was monitored by counting the radioactivity left after incubation on test filters to which high specific activity ^{14}C-DNA from *Xenopus* culture cells had been added. Hybridisation conditions were 2×SSC, 50% formamide, 45 °C, 16 h. *b*, Competition hybridisation curves obtained when increasing concentration of 'cold competitor rRNA' was added to an annealing mixture containing a constant amount (1.5 µg) of ^{32}P-18S+28S ribosomal RNA from *Xenopus* tissue culture cells. Conditions were as in (*a*) except that the specific activity of the ^{32}P-rRNA was 3.4×10^5 c.p.m. per µg RNA. ■, Unlabelled competitor *Xenopus* 18S+28S RNA; □, ^{32}P-18S +28S RNA. For hybridisation, whole or monomer DNA was prepared as described in the legend to Fig. 1. DNA determinations were by the method of Burton[46]. Whole, undigested DNA was sheared by either repeated passages through a 26 gauge needle or by homogenisation in a Virtis S23 homogeniser at full power for 1 min. Nuclease-derived DNA fragments were not sheared. For hybridisation experiments, the membrane filter technique described by Gillespie[34] was used. The purified DNA dissolved in 1/10×SSC at 100 µg per ml was heat denatured at 95 °C for 15 min. The denatured solution was cooled quickly in a dry-ice acetone bath and diluted with cold 6×SSC to a concentration of less than 10 µg per ml. The 6×SSC–DNA solution was made 0.05 M in KOH and 1 mM in MgCl$_2$ to increase the retention of small DNA fragments to nitrocellulose filters. Millipore HA filters (45 mm) were washed with hot (90 °C) 6×SSC, cooled and then loaded with the ice-cold single-stranded DNA solution by slow gravity filtration at 4 °C. Gravity filtration was repeated twice. The loaded filters were then treated for 1 h at room temperature with Denhardt's[47] preincubation mixture, dried and baked overnight at 60 °C. From each 45-mm HA filter, small 6-mm minifilters (each containing 5–10 µg of DNA) were cut. After annealing of DNA filters with radioactive RNA as described, the RNA–DNA hybrid-containing filters were washed extensively with 2×SSC followed by incubation for 15 min at 37 °C in 2×SSC containing boiled RNase (50 µg ml^{-1}). The filters were then dried and counted in an Isocap scintillation counter. All counts on filters were corrected for background and for channel spill over where two isotopes were used. To purify isotopically labelled RNAs, exponentially growing tissue culture cells in low phosphate medium were labelled for 3 d with ^{32}P as orthophosphate (New England Nuclear) and then chased for 48 h. Alternatively, cells in normal medium were labelled similarly with ^{3}H-uridine (New England Nuclear). Whole cell RNA was extracted and separated into fractions by polyacrylamide gel electrophoresis as described by Miller and Knowland[33]. Segments of the gels corresponding to either the 28S ribosomal RNA alone or to the 18S+28S rRNAs together were cut from the gels. The RNA was recovered from these gel fragments by electrophoresis against a dialysis membrane followed by ethanol precipitation. Radioactive 5S RNA, on the other hand, was recovered from the polysome fraction of ^{32}P-labelled tissue culture cells. RNA was extracted from polysomes and separated by gel electrophoresis as described above. The 5S RNA was further purified by chromatography on a Pasteur pipette column containing methylated albumin kieselguhr using a 0.2–1.5 M NaCl gradient for development[48].

Agarose slab gel the results shown in columns b–e of Fig. 1 are obtained. As expected, the peaks on the gradient correspond primarily to DNA fragments of increasing molecular weight. There is, however, always some minor cross contamination of all of the gradient-separated DNA bands. Nevertheless, by sucrose gradient separation it is possible to obtain greatly enriched populations of chromatin particles of a particular size class for extraction of DNA fragments for hybridisation experiments. The same experiments have also been done with DNA fragments of various sizes isolated by elution from Agarose gels after electrophoretic separation. The annealing results are similar in both cases.

Hybridisation with DNA from transcriptionally active cells

The reiterated ribosomal cistrons of *X. laevis* comprise about 0.2% of the nuclear DNA of diploid somatic cells[25,26]. This corresponds to 450–500 adjacent sets of ribosomal genes per haploid genome[25–27]. Each of these tandemly repeated gene sets, in turn, includes DNA which codes for a precursor to

18S and 28S ribosomal RNA as well as DNA for the untranscribed 'spacer' regions of high deoxyguanylic and deoxycytidylic acid (G+C) content[28,29]. Thus the DNA from wild-type animals contains about 1,000 sets of these ribosomal cistrons per diploid genome. Within the cell nucleus of such wild-type animals there are usually two nucleoli (hence they are +/+ nu) with each nucleolus containing about half of the total ribosomal cistron sets localised to a nucleolar organiser region of one of the chromosomes[30]. There is also a stable mutation, the anucleolate mutation[31] known in certain strains of these animals which involves the total deletion of the reiterated cistrons from the normal nucleolar organiser region of the chromosome[30]. Animals heterozygous for this mutation (+/0 nu) contain only half the normal complement of ribosomal cistron sets and have only one nucleolus per diploid somatic cell. Individuals homozygous for the anucleolate mutation (0/0 nu) have no detectable ribosomal 18S and 28S cistron sets[30] and die at an early larval stage. DNA isolated from either +/+ nu, +/0 nu or 0/0 nu embryos was used as reference standards for the nucleic acid hybridisation studies reported here.

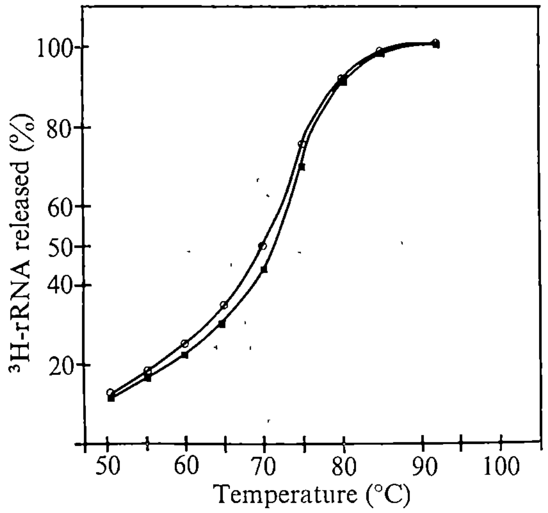

Fig. 3 Thermal dissociation profiles of rRNA–DNA hybrids of sheared whole DNA (○) and nuclease-derived monomer DNA (■) from actively growing tissue culture cells. Filters containing (28S+18S)RNA–DNA hybrids were heated in 1 ml of 0.1×SSC for 5 min at each temperature, and the radioactivity released was measured after precipitation with cold trichloroacetic acid. At no temperature were the ^{3}H counts released from the filters able to rebind to other membrane filters, showing that intact hybrids were not released[34]. Line codes as labelled.

Figure 2*a* gives the results of saturation hybridisation experiments with purified 18S + 28S ribosomal RNA annealed to DNA immobilised on nitrocellulose filters in the low temperature-formamide hybridisation conditions of McConaughy *et al.*[32]. Detailed hybridisation information is given in the legend to Fig. 2. The hybridisation conditions chosen (2×SSC, 50% formamide, 45 °C) correspond to incubation at 25 °C below the T_m of *Xenopus* rRNA–DNA hybrids and ensures both a high specificity of annealing and fast reaction rate[33].

The specificity of the hybridisation reactions reported on here are indicated by the following findings: (1) there is only a slight reduction in the number of RNA–DNA hybrids retained on a filter after RNase treatment to eliminate partially paired hybrids[34]; (2) reproducible saturation plateaux are attained after ribonuclease treatment of the hybrids; (3) unlabelled ribosomal RNA competes effectively with isotopically labelled rRNA in annealing experiments (Fig. 2*b*); (4) there is high thermostability of both sheared whole DNA–rRNA hybrids and chromatin monomer DNA–rRNA hybrids (Fig. 3) with T_m values in agreement with those reported by others[26,35,36] in the same salt conditions; and (5) the saturation plateaux obtained with hybridisation of 18S and 28S ribosomal RNAs to DNAs from +/+ nu, +/0 nu, and 0/0 nu animals are within the range of expectation[27,30,33].

Figure 2 shows the results from one hybridisation experiment in which all DNA filters were annealed with increasing concentrations of the same batch of ^{32}P-labelled RNA in identical hybridisation conditions. Figure 2*a* shows that undigested DNA isolated from wild-type (+/+ nu) embryonic tissue culture cells reaches a saturation plateau of about 0.056% of the DNA hybridised even at low concentrations of input ^{32}P-rRNA (that is, at about 1.5 μg per ml of RNA). On the other hand, DNA from 0/0 nu embryos homozygous for the anucleolate mutation does not hybridise above the level of heterologous *E. coli* DNA, confirming the absence of detectable ribosomal cistrons in these embryos. DNA from heterozygous +/0 nu embryos has an intermediate saturation level between these two at about 0.032% hybridised corresponding to about 550 copies of the 18S and 28S ribosomal cistrons per cell. As is evident, the saturation plateaux for monomer, dimer, trimer and tetramer (and larger) DNA fragments fall above this heterozygote level but do not reach the level of hybridisation of undigested wild-type DNA. In addition, the saturation plateaux for longer pieces of DNA (in dimers, trimers and so on) are seen to reach increasingly higher levels compared with that of the monomer DNA. The plateau level for 18S+28S rRNA annealed to monomer DNA from exponentially growing tissue culture cells averages about 0.043% of the DNA hybridised (from four experiments). This represents about 72% of the level of hybridisation for bulk undigested DNA from the same cells. A similar reduction in the number of ribosomal cistrons in monomer DNA is obtained if hybridisation experiments are done with purified 28S ribosomal RNA alone (Table 1). Again, only about 73% of the number of 28S ribosomal genes present in sheared whole DNA are found in monomer DNA. These results most probably indicate that 28S and 18S ribosomal DNAs are both equally accessible to nuclease digestion in these transcriptionally active tissue culture cells.

As Fig. 2*a* shows, larger pieces of nuclease-cleaved DNA seem to contain greater numbers of rDNA fragments than do smaller pieces. Exact quantitation of the relative numbers of cistrons present in these increasingly long oligomers is difficult,

Table 1 Saturation hybridisation of DNA from logarithmic phase embryonic tissue culture cells with purified 28S and 5S ^{32}P-RNA

RNA*	DNA	μg of RNA per μg of DNA ×100±s.d.§	Mean no. of gene† copies per genome	No. of genes‡ as % of wild type
28S	Undigested	0.041±0.002	982±48	100
28S	Monomer	0.030±0.003	718±72	73
5S	Undigested	0.055±0.005	56,485±5,130	100
5S	Monomer	0.040±0.008	45,170±8,200	80

*Specific activity of ^{32}P-28S RNA was 6.4×10^5 c p.m. per μg RNA; of ^{32}P-5S RNA, 1.1×10^6 c.p.m. per μg RNA

†Calculations based on assumption that each somatic diploid cell contains about 6 pg of DNA[26,29] and that 28S RNA has a molecular weight of 1.5×10^6 and 5S RNA a molecular weight of 35,000 (refs 26, 27, 35 and 36).

‡The assumption is made that a weight of monomer DNA equivalent to the weight of DNA in a normal somatic cell is a genome equivalent.

§Standard deviations based on four experiments.

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Table 2 Saturation hybridisation of adult erythrocyte DNA with ^{32}P-(28S+18S) RNA and ^{32}P-5S RNA

RNA*	DNA	µg of RNA per µg of DNA ×100±s.d‡	No. of genes† as % of wild type
28S+18S	Undigested	0.058±0.002	100
28S+18S	Monomer	0.050±0.004	86
5S	Undigested	0 052±0.002	100
5S	Monomer	0.046±0.004	89

*Specific activity of the 28S+18S ^{32}P-RNA was 8.8×10^6 c.p.m. per µg RNA; of the ^{32}P-5S RNA, 1.2×10^6 c.p m per µg RNA
†Assumptions as in Table 1.
‡Standard deviations based on either three or four experiments in each case.

however, because of the limitations of sensitivity of nitrocellulose filter hybridisation reactions.

Hybridisation using transcriptionally inactive cells

The mature nucleated erythrocytes of *X. laevis* are, as are most vertebrate erythrocytes, transcriptionally quiescent and do not synthesise detectable amounts of rRNA[37].

Chromatin from adult red blood cells was thus chosen for comparison with the transcriptionally active culture cells to determine whether there are detectable differences, in terms of accessibility to nuclease digestion, between synthetically active and inactive genes. The results of hybridisation experiments using 18S+28S rRNA or 5S RNA to saturate either bulk or monomer DNA from adult erythrocytes are given in Table 2.

The saturation levels for hybridisation of both types of RNA to undigested bulk erythrocyte DNA are similar to the plateaux for the same types of RNAs annealed to bulk culture cell DNA (Fig. 1 and Table 1). Erythrocyte monomer DNA (either obtained from gradients or eluted from gels after electrophoretic separation), however, consistently gave higher annealing plateaux for both the 18S+28S and 5S RNA populations than did monomer DNA derived from the synthetically active culture cells (compare Fig. 1 and Table 1). Thus an average of 86% of the 18S+28S ribosomal cistron DNA was protected from degradation in the erythrocyte chromatin subunits compared with about 72% being protected in the culture cell monomers. Similarly, close to 90% of the DNA coding for 5S RNA was recovered in blood cell monomers compared with only about 80% from embryonic cell subunits.

Structure of chromatin

The hybridisation results reported here support the notion that there is no simple, straightforward relationship between chromatin accessibility to micrococcal nuclease digestion and the transcriptional activity of the chromatin itself[16–21]. Most reiterated ribosomal cistrons and 5S genes are associated with protective proteins in the form of subunit particles whether the genes are being actively transcribed or not (Fig. 1, Tables 1 and 2). On the other hand, even in the chromatin of transcriptionally quiescent erythrocytes there are regions or stretches of DNA comprising up to 10–15% of the total reiterated gene copies that can be cleaved by nuclease attack (Table 2). Whether these 'open' regions of DNA in the inactive red cell chromatin reflect the fact that 10–15% of each of the multiple gene copies present are accessible to digestion or simply mean that 85–90% of all of the ribosomal genes are completely protected by monomer proteins cannot be determined from the data.

A similar ambiguity exists when considering the data presented in Fig. 1 and Table 1, showing that there are fewer ribosomal gene sequences protected from nuclease digestion in the chromatin of transcriptionally active cells than in chromatin that is not active in synthesis of rRNA. These data could be construed to mean that transcriptionally active DNA is more 'free' or 'open' than inactive DNA. But there are several technical and theoretical uncertainties, perhaps the foremost being the difficulty of interpreting hybridisation data concerning the transcriptional activity of genes that are present in multiple copies within a genome. For instance, it is not known what fraction of the total number of reiterated genes is functional at any given time within a random population of growing cells. Even though RNA is synthesised continuously throughout the cell cycle (except in mitotic cells)[38], it is not at all clear that all of the genes coding for rRNA need be active in transcription at the same time.

An example of this latter problem for the somatic cells of *X. laevis* comes from the work of Knowland and Miller[33,39]. These investigators have demonstrated that the absolute rate of ribosomal RNA synthesis is the same in wild-type (+/+ nu) and 0/+ nu embryos even though the latter embryos had only half the normal complement of ribosomal cistrons. These results were interpreted to mean that either the 0/+ nu embryos had doubled their rate of transcription of ribosomal genes or that the same number of active rRNA genes were present in both wild-type and mutant animals.

This report thus lends experimental support to a model of chromatin structure in which most of the DNA within genes is associated with proteins to form a 'chromatin subunit' whether or not the genes are actively involved in transcription. The data can also be interpreted to support the view that, during active transcription, genes may have all or some parts of their DNA in a more open or free configuration so as to allow ready access to heterologous molecules, such as experimentally applied nucleases. As discussed, however, this latter interpretation is still a matter of speculation. A direct test of this hypothesis might be to determine the nuclease accessibility of +/0 nu embryo chromatin (in which all of the ribosomal genes must be active for survival) before and after the onset of ribosomal RNA synthesis at the gastrula stage of development[40]. Such experiments are in progress.

We thank Dr J. B. Gurdon for +/0 nu animals and Dr H. Boyer for restriction pieces of SV40 DNA. We also appreciate many helpful discussions of Drs D. Baillie, P. Candido, B. Honda, F. Wilt and H. Woodland. This work was supported by a grant from the National Research Council of Canada.

Received October 17, 1975; accepted February 2, 1976.

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Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene

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Bacteriophage MS2 RNA is 3,569 nucleotides long. The nucleotide sequence has been established for the third and last gene, which codes for the replicase protein. A secondary structure model has also been proposed. Biological properties, such as ribosome binding and codon interactions can now be discussed on a molecular basis. As the sequences for the other regions of this RNA have been published already, the complete, primary chemical structure of a viral genome has now been established.

RNA BACTERIOPHAGES such as MS2 have been important in molecular biology not only because they provide a model system for investigating viral RNA replication and the physiology of the infected cell, but also in the study of fundamental processes such as translation[1]. MS2 RNA contains the genetic information to specify three viral polypeptides (Fig. 1). The first two genes code for the A protein and the coat protein, which are structural elements: the virus particle contains one A-protein molecule[2] and approximately 180 coat protein molecules. For practical reasons, most knowledge of the *in vitro* replication of phage RNA is based on work with a purified system derived from cells infected with the distantly related bacteriophage Qβ (reviewed in ref. 3), but the principal findings are presumably also valid for the MS2 phage group[4]. The enzyme complex responsible for viral RNA replication has been referred to variously as viral RNA-dependent RNA polymerase[5], RNA synthetase[6] and replicase[5]. As the latter name is simple and unambiguous we use it here. The replicase complex consists of four polypeptides, α, β, γ and δ[4,7,8]. β is specified by the third and last viral gene and has a molecular weight of approximately 63,000 (ref. 4). α has been identified as the 30S ribosomal subunit S1 (ref. 9) and γ–δ as the elongation factors Tu–Ts[1,3].

The expression of the three viral genes is strictly regulated. The A-protein gene is presumably only translated from chains in a nascent state[1,10,11]. The replicase gene is subject to a polar control by the coat gene; this means that the latter has to be translated to allow expression of the replicase gene[1,10]. Moreover, the coat protein represses the replicase gene[1,10] while the replicase complex in turn might be involved in shutting off coat gene expression[12]. Regulation of the frequency of gene expression by modulation has also been proposed[13,14].

We have shown that the MS2 genome starts from the 5′ end with a 129-nucleotide untranslated leader sequence (Fig. 1)[15]. Then follows the A-protein gene which starts with a G–U–G initiation codon and ends with a U–A–G codon[11,16], an intercistronic region of 26 nucleotides, the coat protein gene[14], an intercistronic region of 36 nucleotides[14,17], the replicase gene (see below) and finally a 174-nucleotide untranslated segment at the 3′ terminus[16,18]. The proposed secondary structure models provide a rational basis for explaining such biologically relevant phenomena as the autocontrol of A-protein gene expression[11], the polarity effect[14] and the location of easily mutable sites[11,14].

We now report the complete primary structure of the third and last MS2 gene, which codes for the replicase subunit. Some partial nucleotide sequences have been published before[13,19,20].

Nucleotide sequence and structure

Our methods, which have been described before[11,16,18], were briefly as follows. Uniformly ^{32}P-labelled MS2 RNA (2–8 mCi) was digested partially at 0 °C with the single-strand-specific ribonuclease T_1, and the digest was fractionated by electrophoresis on a polyacrylamide slab gel[15]. A series of bands was obtained, and the material in each band was separated further into individual fragments by two-dimensional gel electrophoresis[21]. The advantages of the latter method are that, in general, pure and unique fragments are obtained and that the procedure is applicable to virtually any chain length. These pure fragments were suitable for detailed sequence analysis, mainly according to the methodology described by Sanger and colleagues[22,23], or modifications thereof[15,24]. The structural determination of the longer oligonucleotides required various methods[25,26] and will be described in detail elsewhere.

Discrete fragments can be obtained reproducibly because of the specific three-dimensional conformation of the viral RNA; for example, a fragment may correspond to a hairpin. In milder conditions of partial enzymatic digestion, larger fragments are obtained, for example, several hairpins linked to each other or bound by secondary interactions. Although in this way several regions can often be ordered, this approach has practical limits. On the one hand, if a fragment becomes too large (for example, more than 300–400 nucleotides) its oligonucleotide composition can no longer be established accurately and on the other hand, some sites in the viral RNA are extremely sensitive to T_1 ribonuclease (see legend to Fig. 2). To solve this problem, we introduced the use of carboxymethylated ribonuclease (CM ribonuclease); this enzyme cleaves mainly between C and A

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and between U and A residues[27]. Such partial digestion of MS2 RNA with CM ribonuclease identified a complete new set of fragments. These not only often constituted suitable material for solving nucleotide regions not well represented in T_1 fragments, but by and large provided all the essential overlaps, which have enabled us to reconstruct the entire sequence.

For identification of the fragments and for correlating the T_1 fragments with the CM ribonuclease fragments, we used a catalogue of all the unique oligonucleotides (mainly T_1 oligonucleotides). In this respect, an improved minifingerprinting system[28] has been very helpful in the last stages of this work.

The nucleotide sequence of the replicase gene is shown in the form of a secondary structure model in Fig. 2. It starts with the initiation codon A–U–G and ends with the termination codon U–A–G; these regions have been published before[14,16,18]. Figure 2 also shows sites which are sensitive to ribonuclease T_1, but which could be bridged by suitable CM-ribonuclease fragments, and vice versa for sensitive CM sites. For example, the T_1 fragment 2381–2532 was nearly always found in the same band of the primary gel separation system as the T_1 fragment 2533–2631, but no linkage in primary sequence could be demonstrated. Direct proof for the correct order, however, was provided by the characterisation of several CM fragments, such as 2500–2596.

Such fragments, clearly linked by secondary interactions and only separated in the denaturing conditions of the first dimension of the two-dimensional gel fractionation system[21], provided the primary basis for the construction of the secondary structure model. Sometimes a unique pairing scheme could be derived, but more often there were several alternatives (usually varying only in detail). On the basis of simplified rules for estimating thermodynamic stability[29,30], the most plausible configuration can be selected. After these hairpins have been fixed, additional, thermodynamically stable interactions can then be sought. The model shown in Fig. 2 is much more tentative as far as the latter aspects are concerned. But it is satisfying that by and large the feathered arrows, which are a measure of the sensitivity of the site towards nucleases, point to loops at the top of hairpins, or to discontinuities in the helical segments or to other single-stranded loci. Further study of the model by chemical modification is in progress.

Some of the segments, which remained single stranded in the model shown in Fig. 2, can be base paired to other regions of the molecule (Fig. 3). Such long distance interactions (that is, linkage of segments far apart in the primary sequence) certainly agree with hydrodynamic and other physical properties of MS2 RNA[31,32], but they are not supported by direct experimental evidence. Some of these interactions may have important functional roles, as in the control of the genetic expression or in encapsidation. For example, the coat protein gene must be translated before the ribosome-binding region of the replicase gene becomes accessible. This polar effect can be explained by the interaction of segment 1409–1433 with segment 1738–1769 (Fig. 3), as proposed in a slightly different form[14]. This does not exclude that rearrangements to other conformations may occur[14,33].

Little can be said about the tertiary interactions of the type found in tRNA[34], which almost certainly are also present in MS2 RNA. Yet they may influence profoundly the structure–function relationship. Moreover, some alternative conformations which have not been retained in the present model (Fig. 2) may be stabilised by such tertiary interactions and thus may represent the true conformation.

Sequence of replicase subunit

On the basis of the nucleotide sequence, we can deduce the whole amino acid sequence of the replicase gene product (Fig. 4); only the first three amino acids had been established previously by direct peptide analysis[35,36]. This is the first protein for which the primary structure has been solved entirely on the basis of the genetic information which encodes it.

The replicase subunit is a 544-amino acid polypeptide. Like the A protein, it ends with an arginine residue. It is even more rich in arginine than the A protein (some arginine residues may be involved in interactions with the RNA). But as the aspartic acid content is also high (6.0%), the polypeptide is slightly less basic than the A protein. Also noteworthy are a relatively high leucine content (9.0%; also high in the A protein) and phenylalanine content (6.9%). On the other hand, valine, alanine and glutamine are low, compared with the two other viral proteins.

At present no very meaningful deductions can be made from the amino acid sequence. With available procedures it is not possible to derive a plausible conformation and even less to interpret the structure–function relationship. But when more

a

	U	C	A	G	
U	Phe 12	Ser 7	Tyr ⑤	Cys 5	U
	16	12	16	2	C
	Leu 8	6	Ochre	Opal	A
	⑤	10	Amber o	Trp 9	G
C	Leu 7	Pro 10	His ④	Arg 11	U
	15	4	⑥	13	C
	8	3	Gln 7	④	A
	⑦	9	8	⑧	G
A	Ile 7	Thr 4	Asn 11	Ser ④	U
	13	12	7	9	C
	⑫	⑧	Lys 9	Arg ④	A
	Met ⊙+9	7	16	②	G
G	Val 9	Ala 15	Asp 19	Gly 19	U
	10	7	14	7	C
	6	8	Glu 9	8	A
	7	12	13	10	G

b

	U	C	A	G	
U	Phe 19	Ser 15	Tyr 9	Cys 6	U
	29	20	32	6	C
	Leu 17	16	Ochre 1	Opal	A
	11	22	Amber 2	Trp 23	G
C	Leu 15	Pro 17	His 6	Arg 21	U
	26	10	9	20	C
	15	9	Gln 17	10	A
	9	13	22	11	G
A	Ile 12	Thr 19	Asn 17	Ser 8	U
	25	21	28	16	C
	19	13	Lys 19	Arg 7	A
	Met 2+18	14	26	6	G
G	Val 21	Ala 26	Asp 28	Gly 37	U
	21	21	22	16	C
	16	21	Glu 16	12	A
	1+18	23	28	16	G

Fig. 1 The genome of MS2 contains three genes, shown in blocks Untranslated regions are present at both ends of the viral RNA and between the genes. The length of the different regions, expressed in number of nucleotides, is shown on top. The nucleotides in the viral RNA are numbered from the 5′ end to the 3′ end and the positions of some important signals are indicated underneath (the initiation codon is considered part of the genes and the termination codon part of the untranslated region).

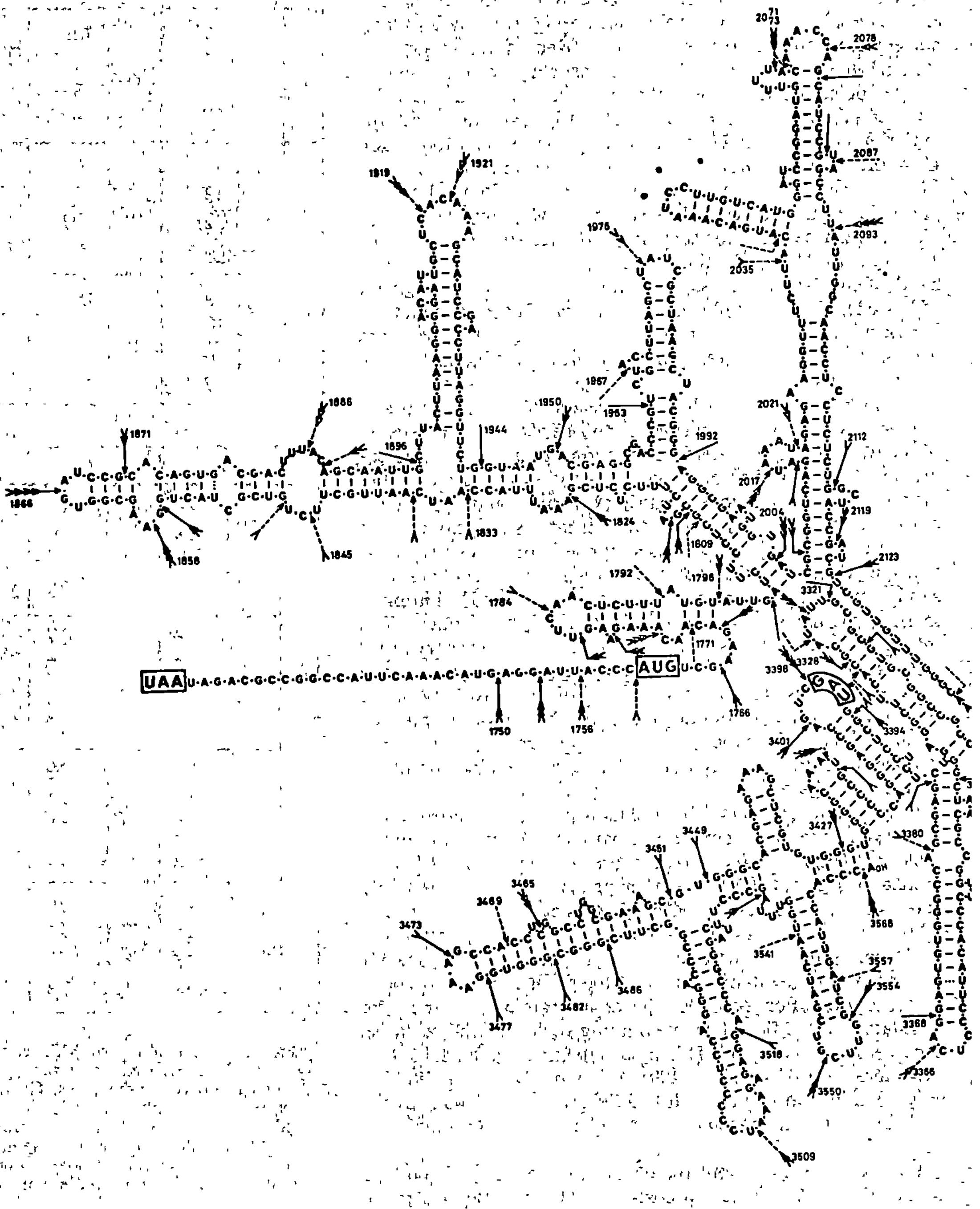
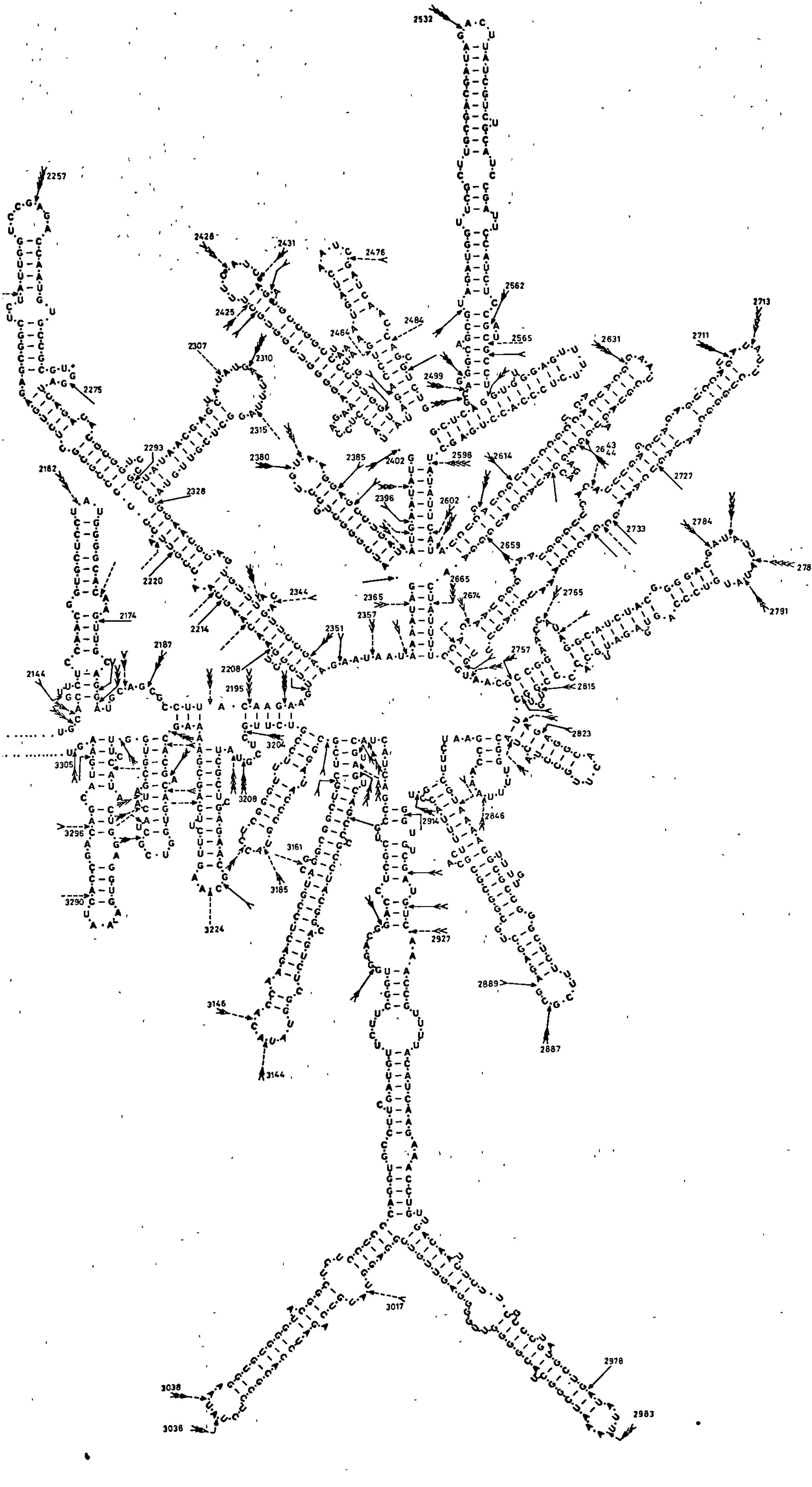


Fig. 2 Primary nucleotide sequence and secondary structure model of the replicase gene. The sequence is read from the 5′ to the 3′ end: U–A–A, termination signal of the preceding coat gene; an intergenic region of 33 nucleotides; A–U–G, the initiation codon of the replicase gene; the replicase gene (1,632 nucleotides); U–A–G, the termination signal; an untranslated, 171-nucleotide 3′-terminal sequence. Together with previous models for the 5′-leader sequence–A-protein gene[11] and the coat gene[14] this figure completes the chemical structure of the entire MS2 RNA. The numbering starts at the first (5′) nucleotide and ends at the 3′-terminal residue 3569. The primary structure has been established rigorously but the secondary structure is based in part on experimental evidence and theoretical predictions and in part on conjectures (see text). Arrows point to sites easily split by nucleases during partial digestion of complete MS2 RNA: solid arrows, T_1 ribonuclease; dashed arrows, CM ribonuclease. The feathers indicate the sensitivity of the bond in the conditions used: (0) split very seldom, (1) seldom, (2) rather often, (3) very often, (4) always.



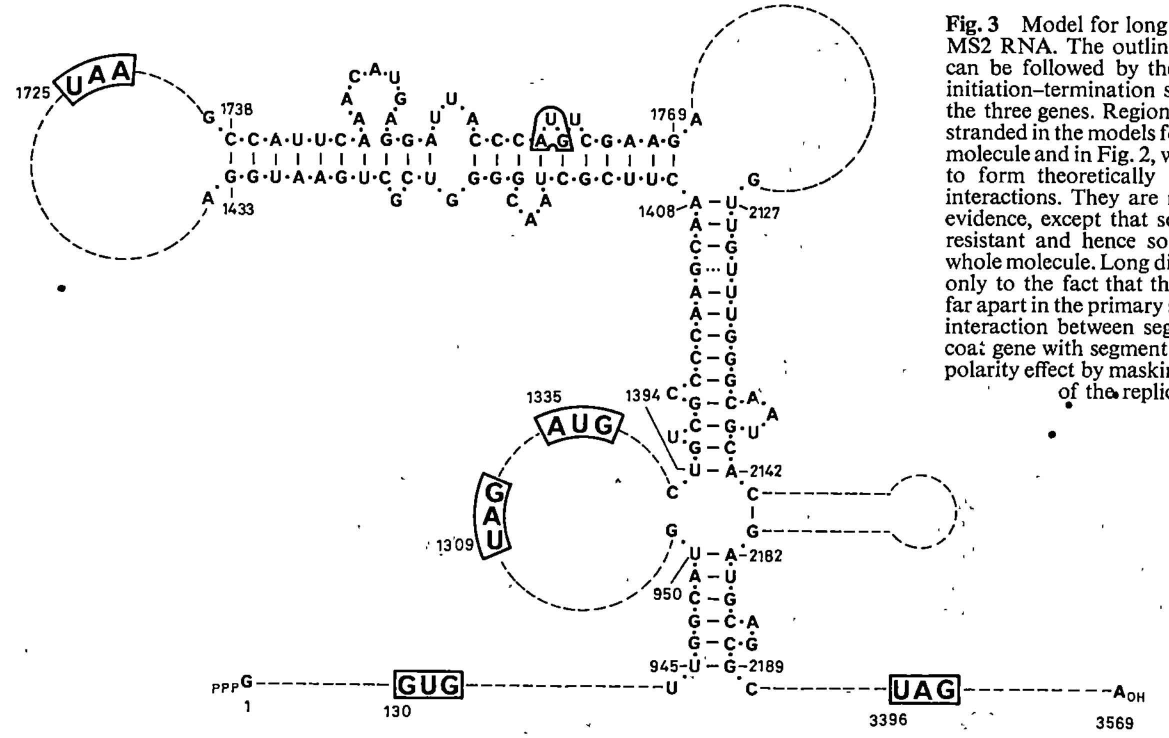


Fig. 3 Model for long distance interactions in MS2 RNA. The outline of the complete chain can be followed by the numbering and by the initiation–termination signals (in bold type) of the three genes. Regions which remained single stranded in the models for the other parts of the molecule and in Fig. 2, were screened for ability to form theoretically stable, complementary interactions. They are not supported by direct evidence, except that some are rather nuclease resistant and hence somehow protected in the whole molecule. Long distance interaction refers only to the fact that the segments involved are far apart in the primary sequence. Note that the interaction between segment 1409–1433 of the coat gene with segment 1738–1769 explains the polarity effect by masking the initiating A–U–G of the replicase gene.

Fig. 4 Nucleotide sequence of the replicase gene and amino acid sequence of the coded polypeptide. As in Figs 1–3, the nucleotides are numbered from the 5′ end of the viral RNA. The amino acid sequence is entirely deduced from the nucleotide sequence data. In analogy with the Qβ polypeptide it is assumed that the initiating formylmethionine has been eliminated in a post-translational process[55]. This polypeptide, together with three host components S1, Tu and Ts, forms a complex which is responsible for specific viral RNA replication.

MS2 REPLICASE GENE

1764
A-U-G U-C-G.A-A-G.A-C-A.A-C-A.A-A-G.A-A-G.U-U-C.A-A-C.U-C-U.U-U-A.U-G-U.A-U-U.G-A-U.C-U-U.C-C-U.C-G-C.G-A-U.C-U-U.U-C-U.C-U-C.
Ser - Lys - Thr - Thr - Lys - Lys - Phe - Asn - Ser - Leu - Cys - Ile - Asp - Leu - Pro - Arg - Asp - Leu - Ser - Leu - 20

1824
G-A-A.A-U-U.U-A-C.C-A-A.U-C-A.A-U-U.G-C-U.U-C-U.G-U-C.G-C-U.A-C-U.G-G-A.A-G-C.G-G-U.G-A-U.C-C-G.C-A-C.A-G-U.G-A-C.G-A-C.
Glu - Ile - Tyr - Gln - Ser - Ile - Ala - Ser - Val - Ala - Thr - Gly - Ser - Gly - Asp - Pro - His - Ser - Asp - Asp - 40

1884
U-U-U.A-C-A.G-C-A.A-U-U.G-C-U.U-A-C.U-U-A.A-G-G.G-A-C.G-A-A.U-U-G.C-U-C.A-C-A.A-A-G.C-A-U.C-C-G.A-C-C.U-U-A.G-G-U.U-C-U.
Phe - Thr - Ala - Ile - Ala - Tyr - Leu - Arg - Asp - Glu - Leu - Leu - Thr - Lys - His - Pro - Thr - Leu - Gly - Ser - 60

1944
G-G-U.A-A-U.G-A-C.G-A-G.G-C-G.A-C-C.C-G-U.C-G-U.A-C-C.U-U-A.G-C-U.A-U-C.G-C-U.A-A-G.C-U-A.C-G-G.G-A-G.G-C-G.A-A-U.G-G-U.
Gly - Asn - Asp - Glu - Ala - Thr - Arg - Arg - Thr - Leu - Ala - Ile - Ala - Lys - Leu - Arg - Glu - Ala - Asn - Gly - 80

2004
G-A-U.C-G-C.G-G-U.C-A-G.A-U-A.A-A-U.A-G-A.G-A-A.G-G-U.U-U-C.U-U-A.C-A-U.G-A-C.A-A-A.U-C-C.U-U-G.U-C-A.U-G-G.G-A-U.C-C-G.
Asp - Arg - Gly - Gln - Ile - Asn - Arg - Glu - Gly - Phe - Leu - His - Asp - Lys - Ser - Leu - Ser - Trp - Asp - Pro - 100

2064
G-A-U.G-U-U.U-U-A.C-A-A.A-C-C.A-G-C.A-U-C.C-G-U.A-G-C.C-U-U.A-U-U.G-G-C.A-A-C.C-U-C.C-U-C.U-C-U.G-G-C.U-A-C.C-G-A.U-C-G.
Asp - Val - Leu - Gln - Thr - Ser - Ile - Arg - Ser - Leu - Ile - Gly - Asn - Leu - Leu - Ser - Gly - Tyr - Arg - Ser - 120

2124
U-C-G.U-U-G.U-U-U.G-G-G.C-A-A.U-G-C.A-C-G.U-U-C.U-C-C.A-A-C.G-G-U.G-C-U.C-C-U.A-U-G.G-G-G.C-A-C.A-A-G.U-U-G.C-A-G.G-A-U.
Ser - Leu - Phe - Gly - Gln - Cys - Thr - Phe - Ser - Asn - Gly - Ala - Pro - Met - Gly - His - Lys - Leu - Gln - Asp - 140

2184
G-C-A.G-C-G.C-C-U.U-A-C.A-A-G.A-A-G.U-U-C.G-C-U.G-A-A.C-A-A.G-C-A.A-C-C.G-U-U.A-C-C.C-C-C.C-G-C.G-C-U.C-U-G.A-G-A.G-C-G.
Ala - Ala - Pro - Tyr - Lys - Lys - Phe - Ala - Glu - Gln - Ala - Thr - Val - Thr - Pro - Arg - Ala - Leu - Arg - Ala - 160

2244
G-C-U.C-U-A.U-U-G.G-U-C.C-G-A.G-A-C.C-A-A.U-G-U.G-C-C.G-C-G.U-G-G.A-U-C.A-G-A.C-A-C.G-C-G.G-U-C.C-G-C.U-A-U.A-A-C.G-A-G.
Ala - Leu - Leu - Val - Arg - Asp - Gln - Cys - Ala - Ala - Trp - Ile - Arg - His - Ala - Val - Arg - Tyr - Asn - Glu - 180

2304
U-C-A.U-A-U.G-A-A.U-U-U.A-G-G.C-U-C.G-U-U.G-U-A.G-G-G.A-A-C.G-G-A.G-U-G.U-U-U.A-C-A.G-U-U.C-C-G.A-A-G.A-A-U.A-A-U.A-A-A.
Ser - Tyr - Glu - Phe - Arg - Leu - Val - Val - Gly - Asn - Gly - Val - Phe - Thr - Val - Pro - Lys - Asn - Asn - Lys - 200

2364

A-U-A.G-A-U.C-G-G.G-C-U.G-C-C.U-G-U.A-A-G.G-A-G.C-C-U.G-A-U.A-U-G.A-A-U.A-U-G.U-A-C.C-U-C.C-A-G.A-A-A.G-G-G.G-U-C.G-G-U.

Ile - Asp - Arg - Ala - Ala - Cys - Lys - Glu - Pro - Asp - Met - Asn - Met - Tyr - Leu - Gln - Lys - Gly - Val - Gly -

220

2424

G-C-U.U-U-C.A-U-C.A-G-A.C-G-C.C-G-G.C-U-C.A-A-A.U-C-C.G-U-U.G-G-U.A-U-A.G-A-C.C-U-G.A-A-U.G-A-U.C-A-A.U-C-G.A-U-C.A-A-C.

Ala - Phe - Ile - Arg - Arg - Arg - Leu - Lys - Ser - Val - Gly - Ile - Asp - Leu - Asn - Asp - Gln - Ser - Ile - Asn -

240

2484

C-A-G.C-G-U.C-U-G.G-C-U.C-A-G.C-A-G.G-G-C.A-G-C.G-U-A.G-A-U.G-G-U.U-C-G.C-U-U.G-C-G.A-C-G.A-U-A.G-A-C.U-U-A.U-C-G.U-C-U.

Gln - Arg - Leu - Ala - Gln - Gln - Gly - Ser - Val - Asp - Gly - Ser - Leu - Ala - Thr - Ile - Asp - Leu - Ser - Ser -

260

2544

G-C-A.U-C-C.G-A-U.U-C-C.A-U-C.U-C-C.G-A-U.C-G-C.C-U-G.G-U-G.U-G-G.A-G-U.U-U-U.C-U-C.C-C-A.C-C-U.G-A-G.C-U-A.U-A-U.U-C-A.

Ala - Ser - Asp - Ser - Ile - Ser - Asp - Arg - Leu - Val - Trp - Ser - Phe - Leu - Pro - Pro - Glu - Leu - Tyr - Ser -

280

2604

U-A-U.C-U-C.G-A-U.C-G-U.A-U-C.C-G-C.U-C-A.C-A-C.U-A-C.G-G-A.A-U-C.G-U-A.G-A-U.G-G-C.G-A-G.A-C-G.A-U-A.C-G-A.U-G-G.G-A-A.

Tyr - Leu - Asp - Arg - Ile - Arg - Ser - His - Tyr - Gly - Ile - Val - Asp - Gly - Glu - Thr - Ile - Arg - Trp - Glu -

300

2664

C-U-A.U-U-U.U-C-C.A-C-A.A-U-G.G-G-A.A-A-U.G-G-G.U-U-C.A-C-A.U-U-U.G-A-G.C-U-A.G-A-G.U-C-C.A-U-G.A-U-A.U-U-C.U-G-G.G-C-A.

Leu - Phe - Ser - Thr - Met - Gly - Asn - Gly - Phe - Thr - Phe - Glu - Leu - Glu - Ser - Met - Ile - Phe - Trp - Ala -

320

2724

A-U-A.G-U-C.A-A-A.G-C-G.A-C-C.C-A-A.A-U-C.C-A-U.U-U-U.G-G-U.A-A-C.G-C-C.G-G-A.A-C-C.A-U-A.G-G-C.A-U-C.U-A-C.G-G-G.G-A-C.

Ile - Val - Lys - Ala - Thr - Gln - Ile - His - Phe - Gly - Asn - Ala - Gly - Thr - Ile - Gly - Ile - Tyr - Gly - Asp -

340

2784

G-A-U.A-U-U.A-U-A.U-G-U.C-C-C.A-G-U.G-A-G.A-U-U.G-C-A.C-C-C.C-G-U.G-U-G.C-U-A.G-A-G.G-C-A.C-U-U.G-C-C.U-A-C.U-A-C.G-G-U.

Asp - Ile - Ile - Cys - Pro - Ser - Glu - Ile - Ala - Pro - Arg - Val - Leu - Glu - Ala - Leu - Ala - Tyr - Tyr - Gly -

360

2844

U-U-U.A-A-A.C-C-G.A-A-U.C-U-U.C-G-U.A-A-A.A-C-G.U-U-C.G-U-G.U-C-C.G-G-G.C-U-C.U-U-U.C-G-C.G-A-G.A-G-C.U-G-C.G-G-C.G-C-G.

Phe - Lys - Pro - Asn - Leu - Arg - Lys - Thr - Phe - Val - Ser - Gly - Leu - Phe - Arg - Glu - Ser - Cys - Gly - Ala -

380

2904

C-A-C.U-U-U.U-A-C.C-G-U.G-G-U.G-U-C.G-A-U.G-U-C.A-A-A.C-C-G.U-U-U.U-A-C.A-U-C.A-A-G.A-A-A.C-C-U.G-U-U.G-A-C.A-A-U.C-U-C.

His - Phe - Tyr - Arg - Gly - Val - Asp - Val - Lys - Pro - Phe - Tyr - Ile - Lys - Lys - Pro - Val - Asp - Asn - Leu -

400

2964

U-U-C.G-C-C.C-U-G.A-U-G.C-U-G.A-U-A.U-U-A.A-A-U.C-G-G.C-U-A.C-G-G.G-G-U.U-G-G.G-G-A.G-U-U.G-U-C.G-G-A.G-G-U.A-U-G.U-C-A.

Phe - Ala - Leu - Met - Leu - Ile - Leu - Asn - Arg - Leu - Arg - Gly - Trp - Gly - Val - Val - Gly - Gly - Met - Ser -

420

3024

G-A-U.C-C-A.C-G-C.C-U-C.U-A-U.A-A-G.G-U-G.U-G-G.G-U-A.C-G-G.C-U-C.U-C-C.U-C-C.C-A-G.G-U-G.C-C-U.U-C-G.A-U-G.U-U-C.U-U-C.

Asp - Pro - Arg - Leu - Tyr - Lys - Val - Trp - Val - Arg - Leu - Ser - Ser - Gln - Val - Pro - Ser - Met - Phe - Phe -

440

3084

G-G-U.G-G-G.A-C-G.G-A-C.C-U-C.G-C-U.G-C-C.G-A-C.U-A-C.U-A-C.G-U-A.G-U-C.A-G-C.C-C-G.C-C-U.A-C-G.G-C-A.G-U-C.U-C-G.G-U-A.

Gly - Gly - Thr - Asp - Leu - Ala - Ala - Asp - Tyr - Tyr - Val - Val - Ser - Pro - Pro - Thr - Ala - Val - Ser - Val -

460

3144

U-A-C.A-C-C.A-A-G.A-C-U.C-C-G.U-A-C.G-G-G.C-G-G.C-U-G.C-U-C.G-C-G.G-A-U.A-C-C.C-G-U.A-C-C.U-C-G.G-G-U.U-U-C.C-G-U.C-U-U.

Tyr - Thr - Lys - Thr - Pro - Tyr - Gly - Arg - Leu - Leu - Ala - Asp - Thr - Arg - Thr - Ser - Gly - Phe - Arg - Leu -

480

3204

G-C-U.C-G-U.A-U-C.G-C-U.C-G-A.G-A-A.C-G-C.A-A-G.U-U-C.U-U-C.A-G-C.G-A-A.A-A-G.C-A-C.G-A-C.A-G-U.G-G-U.C-G-C.U-A-C.A-U-A.

Ala - Arg - Ile - Ala - Arg - Glu - Arg - Lys - Phe - Phe - Ser - Glu - Lys - His - Asp - Ser - Gly - Arg - Tyr - Ile -

500

3264

G-C-G.U-G-G.U-U-C.C-A-U.A-C-U.G-G-A.G-G-U.G-A-A.A-U-C.A-C-C.G-A-C.A-G-C.A-U-G.A-A-G.U-C-C.G-C-C.G-G-C.G-U-G.C-G-C.G-U-U.

Ala - Trp - Phe - His - Thr - Gly - Gly - Glu - Ile - Thr - Asp - Ser - Met - Lys - Ser - Ala - Gly - Val - Arg - Val -

520

3324

A-U-A.C-G-C.A-C-U.U-C-G.G-A-G.U-G-G.C-U-A.A-C-G.C-C-G.G-U-U.C-C-C.A-C-A.U-U-C.C-C-U.C-A-G.G-A-G.U-G-U.G-G-G.C-C-A.G-C-G.

Ile - Arg - Thr - Ser - Glu - Trp - Leu - Thr - Pro - Val - Pro - Thr - Phe - Pro - Gln - Glu - Cys - Gly - Pro - Ala -

540

3384

A-G-C.U-C-U.C-C-U.C-G-G $\boxed{\text{U-A-G}}$

Ser - Ser - Pro - Arg

544

data become available on viral and other RNA polymerases these amino acid sequences may reveal important clues on virus evolution and/or their origin.

Biological information content

The ribosome-binding region, including the initiating A–U–G of the replicase gene, was first identified by Argetsinger-Steitz[37]. Good yields were only obtained after partial unfolding of the viral RNA. It is still not clear which features constitute a ribosome-binding region, although progress has been made. Shine and Dalgarno[38] have proposed that a purine-rich sequence preceding the initiation codon is involved in an interaction with a complementary 3′-terminal segment of the ribosomal 16S RNA. Indeed, all genuine ribosome-binding sites characterised so far contain a sequence of three or more purine nucleotides complementary to the 3′ end of 16S RNA. Also, experimental evidence has been obtained for the direct complementary interaction between the ribosome-binding region and the 3′ end of the 16S rRNA[39]. But this cannot be the whole answer, because many regions in the viral RNA fulfil these criteria, yet are not bound. For example, the segment 2381–2396 is . . . U–A–A–G–G–A–G–C–C–U–G–A–U–A–U–G . . .; according to the criteria mentioned it should interact about as strongly as the A-protein initiation region with both *Escherichia coli* and *Bacillus stearothermophilus* 16S rRNA[38]. That this is not observed may mean that masking/accessibility by secondary and tertiary structure may be equally important. Such cryptic sites may be involved in the additional initiations observed after formaldehyde treatment[40], but these are very unnatural conditions. On the other hand, ribosome binding as tested by protection against nuclease may not be as specific as polypeptide chain initiation. In the original fingerprints of the protected regions of the *E. coli* ribosome there were additional, unexplained spots[37]; on the basis of preliminary identification (J. A. Steitz, personal communication) it seems likely that the main contaminating sequence contained the region 1965–1986. Likewise, in the experiment with *B. stearothermophilus* ribosomes at 49 °C (plate IV in ref. 41) a major contaminant must have been 2926–2967. In neither case is it immediately obvious why this anomalous binding reaction occurred.

Translation of the replicase gene is repressed by the coat protein, and the region to which the coat binds has been characterised[42]. The coat protein may interact with an alternative hairpin structure (Fig. 6 of ref. 14, and ref. 33).

Starting from the initiating A–U–G the nucleotides are read in triplets. For many regions the correct reading frame could be derived independently from the fact that the two illegitimate reading frames were blocked by nonsense code words[19]. The latter occur with a frequency not higher than expected for a random distribution. Finally the reading ends with the terminating U–A–G, as reported before[18]. This assignment is confirmed by studies of specific suppression in an *in vitro* translation system[43]. The replicase gene is followed by a 174-nucleotide untranslated, 3′-terminal segment (including U–A–G); this is shown in a more compact model in Fig. 2.

A heat stable host factor, HF (a hexamer of 72,000 daltons), is required in the Qβ-replicase reaction, but only when Qβ-RNA plus strand is the template[3]. HF binds to two regions in Qβ RNA, one of which is close to the 3′ end[44]. Although it is doubtful that HF is involved in the replication of the RNA of the MS2–R17–f2 group[45], it can nevertheless specifically bind to a single region in these viral RNAs. Senear and Steitz[44] found that HF protects a small segment in R17 RNA against nuclease, which has the following nucleotide sequence: . . . A–A–G–A–A–U–A–A–U–A–A–A–A–U–A–G . . .; undoubtedly this corresponds to the residues 2352–2367 which are located in the middle of the replicase gene. Presumably this interaction has no functional significance; HF is known to have a high affinity for poly(A)[46], and not only is the protected sequence A rich, but the T_1-oligonucleotide A–A–U–A–A–U–A–A–A–A–U–A–Gp has peculiar and very exceptional properties (strongly sticking to cellulose acetate and DEAE paper).

Use of code words

The code words used in the replicase gene are summarised in Fig. 5. On what basis is the choice made between degenerate codons for a given amino acid? In some cases it may be a historical accident, like the choice between a U–C–X or an A–G–Py codon for serine. It is unlikely that there are no constraints on the third letters. Indeed, although the mutation rate is very high (as evidenced by, for example, forward and backward mutation rates), we have been able to work for at least 6 yr with an essentially unaltered sequence. An obvious constraint is that third letters may be involved in optimising the secondary and tertiary structure. Secondary structure can be maximised by a proper choice of third letters and by bringing complementary segments into proper register[47]. Although some evidence in this direction was found[13,14,48,49] in the case of the coat gene, it could not be substantiated further as more data became available[11]. We believe that relatively few, selected third letters are sufficient to bring the molecule from a random secondary structure (50–60% base pairing[49,50]; but this random structure is less stable[51]) to the level of a stable conformation as observed in the viral RNAs (73%±5 base pairing[31]). Statistical methods may not be adequate to reveal these effects convincingly. Moreover, we have no way of assessing the role of third letters in tertiary structure interactions. Neither should we forget that selection may also operate at the level of the negative strand (again no regions should be created which may function in ribosome interaction, or as nuclease targets or as binding sites for encapsidation and so on).

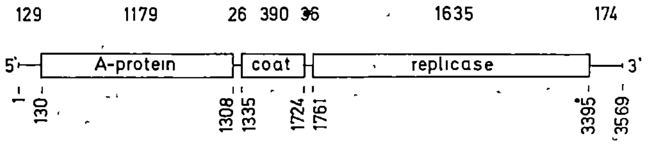


Fig. 5 Code words used in the replicase gene and in the whole genome. The numbers refer to the frequency with which each code word is used. *a*, Codewords used in the replicase gene. Circled numbers indicate codons not used in the coat gene. *b*, Summational table of codewords used in the three viral genes. The initiation codewords (one G–U–G and two A–U–Gs) are counted separately.

But it is very likely that at least for some amino acids other factors directly related to translation in *E. coli* have a predominant role in the choice of the third letter. For example, the code word G–G–U is definitely preferred for glycine and this is true for the whole genome (significant at the 0.1% level as tested by χ^2 analysis). This effect is specific and not due to a general preference for U in third positions, as found in the φX174 gene G[52].

We have suggested that isoleucine, tyrosine and/or arginine have modulating codons. Indeed, in the coat protein the codon U–A–U is not used for tyrosine, and we now conclude that for the whole genome U–A–C is definitely preferred (0.1% significance). Similarly, the code words C–G–Pu and A–G–Pu were not used for arginine in the coat gene, and it now seems that for the whole genome the preference is clearly C–G–Py > C–G–Pu > A–G–Pu (0.1% significance). These modulating triplets may be absolutely unacceptable in a gene like that for the coat, which is translated at high frequency, but can be tolerated in moderate numbers in genes whose elongation rate of translation is average. Isoleucine codons may constitute another class of modulating triplets. A–U–A is absent from the coat gene (5% significance; the coat contains only eight isoleucine residues), and is known to be recognised by a specific tRNA which is present in very small amounts in *E. coli*[53]. The A protein and the replicase genes contain several A–U–A codons, and these may very well modulate (brake) the speed of translation; in fact, in these two genes it seems that it is the A–U–U codon which is selected against (1% significance).

In summary, we have established the primary nucleotide sequence of bacteriophage MS2 RNA. The polynucleotide chain contains 3,569 residues, 10.2% of which constitute untranslated segments. As all the genetic information is derived from this RNA molecule, the primary structure of all the virus-specified products (A protein, coat protein and replicase subunit) has also been deduced. MS2 is therefore the first living organism for which the entire primary chemical structure has been elucidated. We propose tentative models for the secondary folding of the viral RNA; parts of these are based on experimental evidence, and some aspects provide plausible bases for the explanation of biological effects. The secondary structure of the coat gene resembles a flower[14], and there are similar foldings in other parts of the molecule; the secondary structure of the whole viral RNA therefore constitutes a bouquet.

We thank R. Thijs and M. Bensch for technical assistance. This work was supported by a grant from the Belgian Fonds voor Kollektief Fundamenteel Onderzoek.

Received December 24, 1975, accepted February 9, 1976.

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letters to nature

Solar influence on galactic cosmic ray anistropy measurements

COSMIC ray particles of energies $\gtrsim 10^{12}$ eV are virtually unaffected by the interplanetary magnetic field, and thus any galactic anisotropy manifests itself in sidereal intensity variation in Earth-based measurements. Below 10^{11} eV, on the other hand, solar modulation is of importance. Galactic anisotropy would be smeared out at this energy, and an anisotropy is introduced by the solar modulation effects. In the intermediate 10^{11}–10^{12} eV region sidereal variation may originate either in genuine galactic anisotropy or in solar modulation[1]. The London–Torino groups found[2], in accordance with other measurements[3], that, in this intermediate energy range, the phase of the sidereal variation shifted from ~ 1800 to ~ 330 local sidereal time (LST) in 1969 when the polarity of the solar magnetic field reversed. The authors suggested that the change of phase was a result of the change of 'optics': different regions of the sky were scanned before and after 1969, respectively. Here we consider the alternative possibility—that the observed variation was a result of the electromagnetic field embedded in the solar wind.

At energies > 10^{11} eV, the effect of magnetic irregularities is small and particle propagation is governed by the large scale interplanetary magnetic field. Thus, instead of using the transport equations which work well at lower energies, one has to consider individual particle trajectories. Anisotropy at the Earth will be introduced by the energy loss suffered by the particles in the solar system from the electric force associated with the expanding solar wind. If particles lose energy then, at the Earth, we observe the galactic spectrum at higher energy, resulting in a lower flux. As in the London–Torino work[2], we take the magnetic field to be an Archimedean spiral directed away from the Sun everywhere north of the helioequator and towards the Sun south of it for the post-1969 epoch. The exact reverse field is assumed for the pre-1969 epoch. The electric field can be evaluated by requiring

$$0 = \mathbf{E} + 1/c\,(\mathbf{V}\times\mathbf{B}) \qquad (1)$$

for particles comoving with the solar wind velocity **V**. Thus we arrive at the following form of **B** and **E**

$$\begin{aligned} B_r &= B_0(a/r)^2 \quad &; E_r &= 0 \\ B_\theta &= 0 \quad &; E_\theta &= B_0(a/r)(a\Omega/c)\cos\theta \\ B_\varphi &= B_0(a/r)(a\Omega/v)\cos\theta \quad &; E_\varphi &= 0 \end{aligned} \qquad (2)$$

r being the radial distance from the sun, $a = 1$ AU and B_0 being the radial component of the magnetic field at the Earth (2.5 gamma (ref. 2)). θ represents heliolatitude while Ω is the angular velocity of the rotating Sun ($\Omega = 2.7\times10^{-6}\,\mathrm{s}^{-1}$).

There are two implications of equation (2): (1) The electric field is independent of the solar wind velocity; (2) an electric quasi-potential can be introduced with

$$\mathbf{E} = -\nabla\Phi;\ \Phi = aB_0\,(a\Omega/c)|\sin\theta| \simeq 150|\sin\theta|(\mathrm{MV}) \qquad (3)$$

The potential, Φ, exists only within the solar cavity. If the Earth had a well defined potential then the energy loss would be identical for each trajectory, resulting in no anisotropy[4].

In our model, however, the continuous injection of magnetic flux from the solar wind yields a $\nabla \times E$ term, and it is this term which enables cosmic rays of different trajectories to reach the Earth having suffered different energy changes.

Particle trajectories depend only on rigidity, P ($P = pc/Ze$, p being the momentum and Ze being the electric charge of the particle). In the Solar System a highly relativistic particle changes its rigidity by

$$\Delta P = (\mathrm{d}P/\mathrm{d}E)\Delta E = -\Delta\Phi \qquad (4)$$

where $\Delta\Phi$ is the potential difference between the Earth and the point where the particle entered the Solar System. Thus, for a fixed rigidity, P, the relative intensity variation follows as

$$\Delta I(P,t)/I(P) = -(\gamma+2)\,\Delta\Phi(P,t)/P \qquad (5)$$

where γ stands for the negative exponent of the differential rigidity spectrum of the galactic cosmic radiation. $\Delta\Phi(P,t)$ refers to the trajectory reaching the observation site vertically (most of the observed flux arrives nearly vertically). By taking the response function of the detector, $r(P)$, into account we obtain

$$\frac{\Delta I(t)}{I} = -\int_{P_0}^{\infty} (\gamma+2)\,\frac{\partial r(P)}{\partial P}\,\frac{\Delta\Phi(P,t)}{P}\,\mathrm{d}P$$

The detailed analysis of the trajectories carried out by the Imperial College group[5] showed that particles of rigidities $\lesssim$ 300 GV were considerably deflected, while > 300 GV the deflection was small. As a rough approximation, we take that rigidities < 300 GV do not contribute to the intensity variation. Above 300 GV, on the other hand, trajectories are assumed to be straight lines. Obviously the energy change is maximal for the trajectories nearest to the pole (Fig. 1) which results in a sidereal daily variation of 0.017% with times of maximum at 1904 and 0704 LST before and after 1969, respectively. This result is in accordance with the general character of the London–Torino measurement[2] (0.013±0.003%; 1800 for the pre-1969 epoch and 0.012±0.005%; 0330 for the post-1969 epoch).

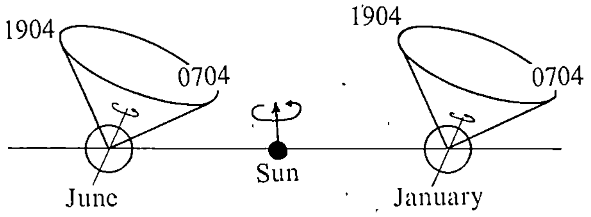

Fig. 1 The cone of the directions scanned for January and June. The maximal energy change occurs for the trajectory nearest to the pole, that is, for 1904 LST.

The model outlined here predicts opposite phases for measurements made on the Northern and Southern Hemispheres, respectively. In a measurement carried out at Hobart[6] between 1957 and 1974 (the pre-1969 interval being dominant) the phase of the sidereal daily wave was found to be 0700 LST, agreeing with this prediction. There was, however, no sign of a reversal of phase in 1969. A detailed investigation of trajectories may give firmer support to the model discussed here or may disprove it. At present, however, one cannot rule out that the sidereal daily variation of cosmic ray intensity in the 10^{11}–10^{12} eV region can be accounted for by solar influence.

I thank Professor H. Elliot and the referee for their remarks on the original manuscript.

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Received October 14, 1975, accepted February 19, 1976.

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Ionising flux of cosmic background radiation

THE intensity of the diffuse cosmic background radiation for photon energies of 13.6 to ~ 150 eV cannot be measured directly because of absorption by the neutral component of the interstellar medium. In the range 150–300 eV, there is considerable galactic emission within a few hundred parsecs of the Sun which places severe limitations on attempts to measure the intensity and spectrum of the metagalactic background radiation at these energies. An indirect means of estimating the diffuse metagalactic flux of ionising radiation was proposed by Sunyaev[1], who studied the interaction of this radiation with neutral hydrogen at the peripheries of galaxies. More locally, the intensity of this radiation within ~1 kpc of the Sun can be probed by studying the ionisation of heavy elements in the interstellar medium[2]. Here we re-examine Sunyaev's proposal in the context of more detailed studies of the interaction of metagalactic ionising radiation with galactic gas. We give a firm upper limit on the intensity of metagalactic ionising background radiation to which the neutral interstellar medium is exposed, and discuss some important consequences of this result. Our principal conclusion is that further than ~30 kpc from the centre of the Galaxy, self-shielding by H II[3,4] is possible only if the flux of ionising radiation is below a certain critical value[5–7].

Hill[6] used a self-gravitating galactic disk model of the galactic gas distribution that was exposed to an ionising photon flux calculated on a variety of possible extrapolations of the high latitude soft X-ray data to lower energies. Bochkarev and Sunyaev[7] used a variety of possible gas density distributions, including disk models without stellar halos, and also models appropriate to a disk embedded in a massive non-gaseous corona.

An important result can be inferred from their studies: there is a critical value of the metagalactic ionising flux; if this value (φ_c) is exceeded, there will be too little neutral hydrogen far from the centre of nearby spiral galaxies. The specific 21-cm observations that we use indicate that, after correcting for projection effects, the H I column density is ~(2–3)× 10^{19} cm^{-2} at ~30 kpc from the centre of M31 (refs 8–11). Now, the hydrogen ionisation rate from hydrogen-primary photo-ionisations at the periphery of the Galaxy is

$$\zeta = 4\pi \int_{\nu_H}^{\infty} \frac{\sigma\varphi}{h\nu}\mathrm{d}\nu \simeq 4\pi\sigma_0\varphi_0\left(\frac{\nu_H}{\nu_0}\right)^{3+\alpha} (3+\alpha)^{-1}\,\mathrm{h}^{-1}$$

where we have written the incident ionising radiation flux (erg cm^{-2} s^{-1} Hz^{-1}) as $\varphi = \varphi_0(\nu_0/\nu)^{\alpha}$. Here $\nu_0(\nu \geqslant \nu_0 \geqslant \nu_H)$ is a low energy cutoff, $\alpha \geqslant 0$, $\sigma_0 = 6.3\times10^{-18}$ cm^2, and ν_H is the frequency at the Lyman limit. It is important to also include the effects of helium recombination radiation and secondary electrons produced by both hydrogen and helium in the exact computations. This gives the critical value[6,7] of ζ as $\zeta_c = 2\times10^{-13}$ s^{-1}, and this in turn gives an upper limit on the metagalactic ionising photon flux > 13.6 eV of $\varphi_c = 5\times10^{-23}(\nu_0/\nu_H)^3$ erg (cm^2 s ster Hz)$^{-1}$ if $\alpha = 0$ (and is readily generalised if $\alpha \neq 0$). ν_0 and α are characteristic parameters of the incident ionising flux: an unattenuated thermal bremsstrahlung spectrum with temperature parameter $kT \gg h\nu_H$ corresponds approximately to $\alpha = 0$ and $\nu = \nu_H$.

This limit depends weakly on the gas distribution; here it is for the model of a self-gravitating infinite disk with surface density varying inversely as galactocentric distance. For other models, somewhat lower limits are obtained that are within a factor of three of this value, which also lies in the same range as Sunyaev's earlier estimate[1].

These results were obtained under the assumption that the gas is diffuse. If there is an intercloud medium which is ionised and heated to $\sim 10^4$ K by the metagalactic ionising radiation, clouds cannot exist in thermally stable pressure equilibrium (and would consequently not be replenished over the cloud dissipation time scale) unless the mean gas density exceeds $\sim 0.03\ \mathrm{cm}^{-3}$ (ref. 5). One remaining possibility would be for H I clouds at the periphery of the Galaxy to be maintained in pressure equilibrium with a tenuous, hot intercloud medium, for example at $T \simeq 10^6$ K and $n \simeq 10^{-3}\ \mathrm{cm}^{-3}$. The low column density of the clouds ($\sim 10^{19}\ \mathrm{cm}^{-2}$), however, renders them transparent to photons of energy $\gtrsim 50$ eV, and the clouds would evaporate over a time scale short compared with the relevant time scale for cloud formation, which we may identify with the cooling time of the ambient medium (particularly if the gas is depleted in heavy elements).

Note that the characteristic propagation time for the ionisation front in the diffuse medium is $\sim 1.7\times 10^7\ (\varphi_c/\varphi)(N/10^{20}\ \mathrm{cm}^{-2})$ yr, where N is the local column density of gas normal to the galactic plane. If φ is sufficiently small, this can exceed the dynamical time scale in the outer regions of the Galaxy; in this case, it is necessary to study the propagation of a non-stationary ionisation front[12].

This conclusion has some notable implications that we now mention. Recent work on the existence of massive galactic coronae[13,14] has indicated that such coronae may contain masses of $\gtrsim 10^{12}\ M_\odot$ within $\lesssim 100$ kpc of the Galaxy. Such coronae could be gaseous and unobservable if the gas temperature were in the range 10^5–10^6 K. Our limit on the ionising radiation flux, however, enables us to impose a severe restriction on the emission from a galactic corona containing gas in this temperature range, and we find that the maximum mass of a gaseous galactic corona (compare ref. 15) is

$$M_{\mathrm{max}} \simeq 1\times 10^{11}(\varphi/\varphi_c)^{1/2}(100\ \mathrm{kpc}/R)^{5/2}(10^{-23}\mathrm{erg\ cm^3\ s^{-1}}/\Lambda^{1/2} M_\odot$$

for a uniform density model, where R is the radius of the corona and $\Lambda(T)$ is the cooling function ($\sim 10^{-21}$–10^{-22} erg cm^3 s^{-1} for $T = 10^5$–10^6 K if the heavy element abundance, Z, is solar; $\Lambda \propto Z$). Consideration of a non-uniform gas distribution only strengthens this limit.

A second result is that we can give an upper limit on the mean luminosity, F, radiated by a QSO in the energy band 40–170 eV. Adopting Schmidt's[16] QSO density distribution function and assuming a non-thermal continuum spectral energy distribution $\propto \nu^{-1}$ (ref. 17), we find from the background limit (in a $\Omega = 1$ cosmological model) that $F \lesssim 10^{29\ 2}$ erg s^{-1} Hz^{-1}; this estimate takes into account the absorption of ultraviolet photons in the neighbourhood of the quasars. Since QSO optical luminosities (at 2,500 Å in the rest frame of the QSO) are in the range 10^{29}–10^{31} erg s^{-1} Hz^{-1} (ref. 16) and several at high redshift are found to be transparent to Lyman continuum photons, we infer that QSO may be expected to make an appreciable contribution to the metagalactic ionising background radiation.

Finally we mention an upper limit on the mean density of intergalactic gas obtained by considering its radiation[1]; the critical flux is exceeded only if the mean density of matter exceeds the value for closure of the Universe when a Hubble constant of 100 km s^{-1} Mpc^{-1} is adopted (note that the radiation of the gas is proportional to $H_0{}^3$).

We realise that this discussion has hitherto been entirely devoted to the astronomy of upper limits. An important observational implication for 21-cm studies of external galaxies is that the presence of a metagalactic ionising background radiation field will affect the distribution of H I in the outer regions of galaxies. The transition between H I and H II occurs over a dimension short compared with the galactocentric distance and with low spatial resolution should be detectable as an abrupt change in H I surface density (compare refs 4 and 5). Because of the hardness of the spectrum of ionising photons, however, the transition zone, in the case of a bremsstrahlung or power-law incident spectrum, can be appreciable, amounting to[4] $\sim 2(\varphi/\varphi_0)\ (0.01\ \mathrm{cm}^{-3}/n)^2\ (T/10^4\ \mathrm{K})^{1/2}$ kpc.

Thus the transition region may be detectable when high resolution 21-cm studies of the outlying parts of external galaxies are available, and would provide useful information on the nature of the ionising photon spectrum.

One of us (J.S.) wishes to acknowledge the hospitality of Professor Ya B. Zeldovich and the USSR Academy of Sciences during the brief period in Moscow when this paper was written. This research was also supported in part by the Alfred P. Sloan Foundation and by NASA.

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New theory of the Great Red Spot from solitary waves in the Jovian atmosphere

THE nature and stability of the Great Red Spot (GRS) on Jupiter have been persistent problems. We show here that solitary waves on a horizontally sheared zonal flow in a rotating, stratified atmosphere can explain many of the GRS characteristics known at present and numerous other features that have been observed on Jupiter (see also ref. 1).

Solitary waves have a number of attractive properties: they are isolated, permanent waves in which nonlinear steepening balances dispersive, spreading effects[2]. They can arise from arbitrary initial disturbances and interact nonlinearly without changing their shape[2]. The only memory of an interaction is a finite spatial phase shift between the post- and the pre-interaction trajectories[2,3], an interaction which looks like a rapid acceleration of one wave through the other.

Their simplest mathematical basis is the quasi-geostrophic potential vorticity equation for an incompressible fluid[4] which, when made suitably non-dimensional and when one assumes a flow composed of a mean zonal shear flow $U(y)$ and a superimposed disturbance $\varepsilon\psi(x,y,z,t)$, has the form

$$[\partial_t + U\partial_x + \varepsilon(\psi_y\partial_x - \psi_x\partial_y)] \times$$
$$\times [\partial^2{}_{xx} + \partial^2{}_{yy} + (f_o l/Nd)^2\partial^2{}_{zz}]\psi + (\beta - U'')\psi_x = 0 \qquad (1)$$

The east–west, north–south and vertical coordinates are x,y,z; t is the time; $f = f_o + (U_o/l)\beta y$ is the local Coriolis parameter;

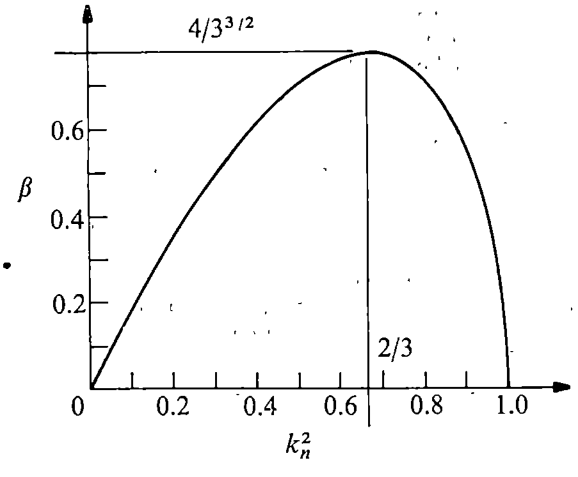


Fig. 1 Eigenvalue relationship for $U=\tanh(y)$; $\beta=2k_n^2(1-k_n^2)^{\frac{1}{2}}$ for $0<k_n^2<1$.

N is the Brunt–Vaisala frequency (assumed constant) and $\mu=d/l$ the ratio of the atmospheric depth to the characteristic zonal wavelength.

Restricting our discussion to a single vertical wave mode, this equation admits a solution of the form

$$\psi=A(\xi,\tau)\varphi(y)\cos(n\pi z)+O(\varepsilon)$$

if $A(\xi,\tau)$ satisfies the modified Korteweg–DeVries equation

$$A_\tau+\alpha A^2A_\xi+\gamma A_{\xi\xi\xi}=0 \tag{2}$$

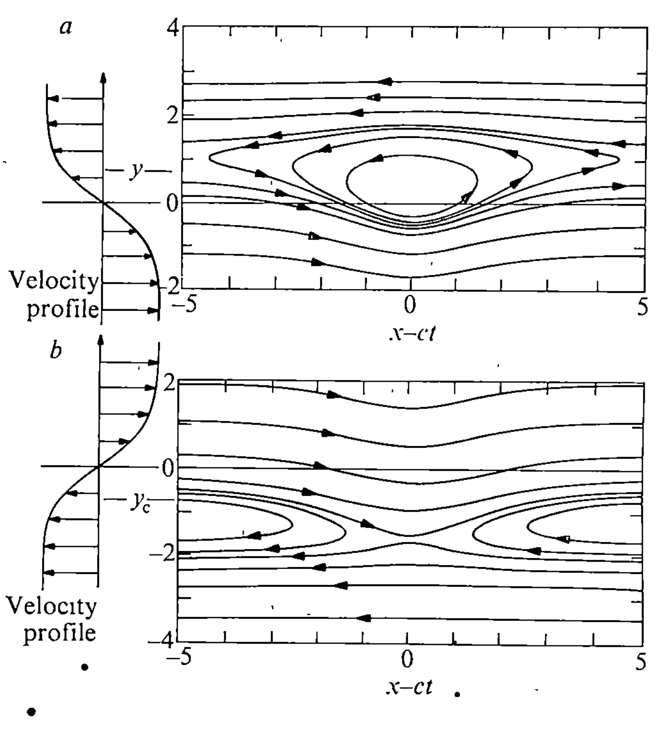
where

$$\xi=\varepsilon(x-c_o t),\quad \tau=\varepsilon^3 t$$

The quantities α and γ are numbers that depend on certain integral properties of φ, its derivatives and $U(y)$. Simultaneously, $\varphi(y)$ satisfies the barotropic stability equation

$$\begin{gathered}\varphi''-k_n^2\varphi+[(\beta-U'')/(U-c_o)]\varphi=0\\ k_n=n\pi(f_o l/Nd)\\ \varphi\to0 \text{ as } |y|\to\infty\end{gathered} \tag{3}$$

Fig. 2 *a*, An E soliton on an anticyclonic shear in the southern hemisphere ($C=-0.7$, $\varepsilon=0.7$). *b*, A D soliton on a cyclonic shear in the southern hemisphere ($C=-0.7$, $\varepsilon=0.7$).



For the shear profile, $U=\tanh(y)$, equation (3) has been solved by Lipps[5]. The appropriate eigenvalue curve for neutral solutions is shown in Fig. 1.

Figure 2*a* and *b* shows typical streamline patterns for waves corresponding to solitary waves of elevation (E solitons) and of depression (D solitons). The first has closed streamlines with pronounced cusps and the second has reversed flow fore and aft of the wave centre. The similarity of the flow shown in Fig. 2*a* to that found around the GRS[6] is obvious and hardly needs further comment. The picture is enhanced when one constructs possible extensions of this theory to the solitary waves one would expect on the asymmetric jet-like profile that exists in the vicinity and equatorward of the GRS. Figure 3 shows a combined D–E soliton that emerges from a combining of flows like those shown in Fig. 2*a* and *b* with distortions arising from their mutual interactions. This picture is even closer to the total flow near the GRS, as shows particularly clearly in Fig. 4

There is strong evidence, both from its morphology and flow pattern (the so-called Circulating Current (Fig. 7, Plate III, Figs 5–9 of ref. 7)) that the South Tropical Disturbance (STD) was a D soliton. When this feature interacted with the GRS, observers noted a very rapid acceleration of the STD and its reformation without a change of shape (ref. 7, 137-143): we noted above that this is how solitary waves interact.

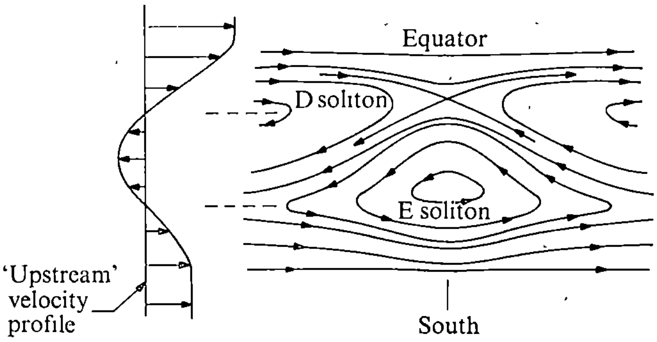


Fig. 3 A combined D–E soliton on an asymmetric jet-like profile

There are a variety of similar examples. The Dark South Tropical Streak (ref. 7, plate IX) has the structure of a D soliton. Again many of the 'spot-like' features in ref. 7, plates V and X, and also in the Pioneer 10 and 11 pictures (the Little Red Spot, for example) sit in regions between cloud bands, where one would expect horizontal shear to exist and have the morphology of E solitons. The 'plume' at the northern edge of the equatorial jet, seen in the Pioneer pictures and the historical record[8], seems to be one branch of a D soliton.

One can also extend, by inference, the present weakly nonlinear theory to a highly nonlinear regime. Then one would expect supercritical flows, with hydraulic jumps, or leewaves, to appear 'downstream' of the solitary waves (as in flow over bodies in rotating or stratified flows). Such flows have been observed around the GRS and can be seen in Fig. 4 downstream of the 'White Oval' below and to the left of the GRS.

The theory also places certain restrictions on the atmospheric parameters, and we now show that these are consistent with available models and observations. Taking a typical point on the eigenvalue curve, $k_n^2\simeq0.8$, $\beta=0.7$ with $U_o\simeq55$ m s^{-1} (ref 6), and a latitude of 24°, we obtain $l\simeq3{,}000$ km (corresponding to a shear zone width for the $\tanh(y)$ profile of 9,000 km) and $Nd/n\simeq1{,}500$ m s^{-1}. For example, an isothermal stratosphere at 110 K has $N=2.2\times10^{-2}$ s^{-1}, in which case $d/n\simeq6.7$ km, or four density scale heights. For more realistic atmospheric models in which N increases from zero, at a depth near the ammonia cloud base, to a maximum at an inversion, our restriction on Nd/n becomes, crudely, one on

$$\int_{0}^{d}(N/n)\mathrm{d}z$$

and more appropriately, we interpret the resulting 'averaged' value of d/n as the vertical wavelength of the wave. In this case the wave now propagates within the cloud layer to a depth at which N is effectively zero (decaying exponentially below this level) and to a height determined by the constraint on

$$\int_{0}^{d}(N/n)\mathrm{d}z$$

For the model of Wallace *et al.*[9], most of the first vertical amplitude oscillation will be in and below the inversion level. Thus, in this model the GRS is not a deep-seated feature, but one that propagates probably no deeper than one scale height below the base of the ammonia cloud layer. In this sense, it differs from the available explanations of Hide[10], Ingersoll[11], Smoluchowski[12] and Kuiper[13], which place most of the motion below the tops of these clouds. Finally, we note that since the flow is related to the corresponding instability of the barotropic shear flow that makes up the basic state, the wave is able to extract energy from this flow and maintain itself in the presence of dissipation, possibly accounting for the long lived nature of the GRS.

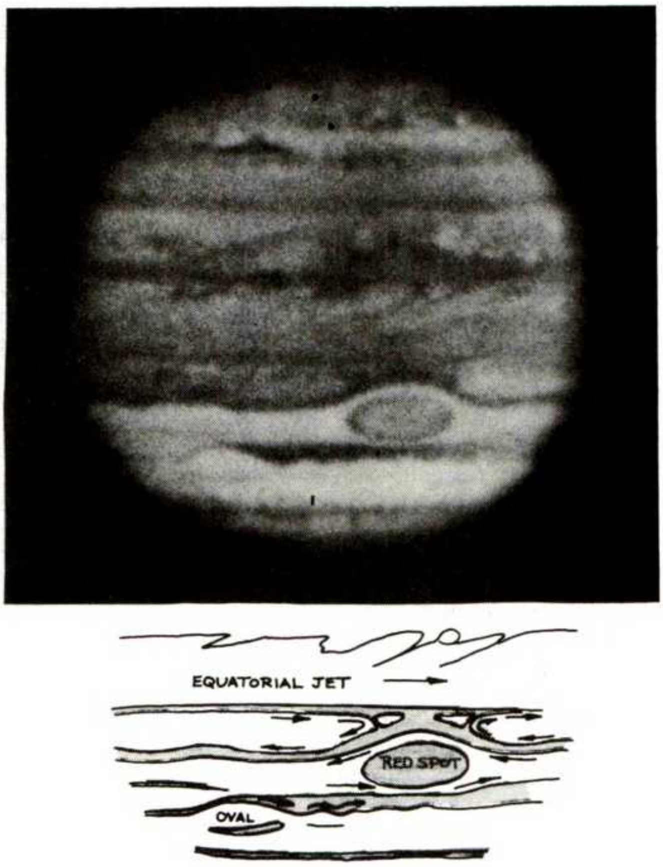


Fig. 4 Photograph taken at Pic-du-Midi Observatory on November 23, 1964 through a yellow filter (photo courtesy of Lowell Observatory) together with an interpretive sketch of the flow field that is consistent with the observations of Reese and Smith[5]. Compared to Fig. 3, this suggests that the GRS and Hollow can be interpreted as a combined D–E soliton. Also note the leewave type of flow downstream of the White Oval below and to the left of the GRS.

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Received July 7, 1975; accepted February 11, 1976.

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Preferential adsorption of ^{137}Cs to micaceous minerals in contaminated freshwater sediment

A MAJOR concern to environmentalists, health physicists and radioecologists is the fate of ^{137}Cs released to the environment from existing light-water power stations (estimated annual discharge of ^{137}Cs as liquid effluent exceeds 2.0 Ci per yr per power station) and future nuclear fuel reprocessing facilities[1]. Its long half life (30 yr) and its ability to be readily assimilated into body tissues make it potentially one of the most hazardous radionuclides for man. Numerous investigations into the fate of ^{137}Cs in terrestrial environments have revealed that soils exhibit a marked capacity to retain ^{137}Cs; thus soils act as a 'sink' for ^{137}Cs released to the environment[2,3], and sediments retain ^{137}Cs in a similar fashion in aquatic environments[4,5].

We report here the detection of preferential adsorption of ^{137}Cs to the micaceous component of a ^{137}Cs contaminated freshwater sediment, containing predominately kaolinite and quartz, using density-gradient mineral segregation in a large scale zonal rotor[10].

Experiments on reference clay minerals have shown that the single most important factor controlling caesium retention is the ability of certain layer silicates such as micas, vermiculites and interleaved micas (formerly called illites) to preferentially adsorb or 'fix' trace quantities of Cs (ref. 6). The 'fixation' mechanisms of these minerals are a result of the trapping of Cs ions in either the interlayer regions of vermiculite, a partially expanded mineral, or at the frayed edges of illites and micas[7]. ^{137}Cs is more strongly retained in soils containing predominately micaceous minerals, thus resulting in considerably less uptake of ^{137}Cs in plants grown on these soils as compared with tropical soils[8], peat soils[8] and acid sandy podzolic soils[9], which do not contain large quantities of micaceous minerals. In no instance before has preferential adsorption of ^{137}Cs to the micaceous component of a soil or sediment containing a variety of minerals been demonstrated in environmental conditions.

Sediment particles from Steel Creek (obtained in a previous experiment[11]) were largely distributed in three ranges: a large band covering the density range from 2.21 to 2.47 g cm^3, a smaller band between 2.47 and 2.57 g cm^{-3} and a band, mostly of quartz, from 2.57 to 2.65 g cm^{-3} (Fig. 1). These bands constituted approximately 48, 22 and 17% of the recovered sediment,

Table 1 Description of the major density ranges of sediment particles fractionated by density-gradient centrifugation in a large scale zonal rotor

Sediment particle density range (g cm^{-3})	Mineral composition* (%)	Fraction by Weight (%)	Fraction by ^{137}Cs activity (%)	^{137}Cs enrichment factor†
<2.21	95 Kl	7.1	5.1	0.72
2.21–2.47	95 Kl, 5 Qz	48.4	60.8	1.26
2.47–2.57	80 Kl, 15 Qz, 5 Mi	21.7	16.1	0.74
2.57–2.65	90 Qz, 5 Kl, 5 Mi	16.8	8.7	0.52
2.65–2.76	85 Mi, 15 Kl	1.9	6.0	3.16
>2.76	Hm	4.0	3.3	0.82

*Kl = Kaolinite, Qz = quartz, Mi = includes interleaved micas and muscovite, Hm = heavy minerals fraction containing anatase, iron-rich chlorite, traces of mica, and iron-rich sesquioxides. The quantities of each mineral were estimated based on their respective X-ray diffraction peak heights.

† Ratio of the fraction of ^{137}Cs activity to the weight fraction.

respectively (Table 1). Before density separation, X-ray diffraction showed only the minerals kaolinite and quartz in the <50-μm fraction (Fig. 2*a*). After fractionation, X-ray diffraction analyses revealed the mineral component in the density range 2.21–2.47 g cm^{-3} to be ~ 95% kaolinite (Fig. 2*b*), whereas the density range between 2.47 and 2.57 g cm^{-3} contained ~ 80% kaolinite and some quartz (~ 15%) and a little muscovite mica (~ 5%). The third major band (density range 2.57–2.65 g cm^{-3}) contained approximately 90% quartz, 5% kaolinite and 5% muscovite mica (Fig. 2*c*). The ^{137}Cs concentrations in these bands are ~ 1.0, 0.6 and 0.4 nCi g^{-1}, respectively. We attribute the decreasing ^{137}Cs concentration with increasing particle density to the increasing concentration of quartz (Table 1).

The highest ^{137}Cs concentration was observed in the particle density range from 2.65 to 2.76 g cm^{-3} (Fig. 1). The ^{137}Cs concentration in this density fraction (maximum >3.0 nCi g^{-1}) was three times that observed in the density fraction 2.21–2.47 g cm^{-3}, whose major mineral was ~ 95% kaolinite. The mineral component in the 2.65–2.76 g cm^{-3} density fraction was ~ 85% muscovite (Fig. 2*d*). The quantity of sediment particles isolated in this density range (2.65–2.76 g cm^{-3}) was only 0.28 g or <2% of the recovered sediment sample. Thus, through the use of density-gradient centrifugation in a large scale zonal rotor, sufficient quantities of the muscovite mica component of the sediment could be separated from the dominant minerals, kaolinite and quartz, so that the ^{137}Cs concentration associated with the micaceous component could be determined. This is the first explicit demonstration that ^{137}Cs is selectively adsorbed to 'Cs-fixing' minerals in environmental conditions.

Approximately 6% of the total ^{137}Cs burden is directly associated with muscovite and >80% is associated with density fractions whose major mineral component is kaolinite (Table 1). Less than 20% of the ^{137}Cs associated with the dominant kaolinite band (2.27–2.33 g cm^{-3}) could be displaced with 2 N CsCl and only 22% could be displaced with 5 N NaCl. On the other hand, 75–83% of the ^{137}Cs could be displaced from reference kaolinite clay using 1 N NaOAc[4], indicating minerals other than kaolinite are responsible for the observed 'fixation'. Delineating the ^{137}Cs 'fixation' mechanism for these kaolinite bands is beyond the scope of this paper. It is conceivable, however, that the quantities of interleaved micas present were sufficient to 'fix' ^{137}Cs, but could not be detected by X-ray diffraction procedures. Interleaved micas, because of their irregular interlayer spacing, are difficult to detect by X-ray diffraction. Soils that contain appreciable quantities of mica in the density range between 2.65 and 2.78 g cm^{-3} also contain interleaved micas that band between 2.21 and 2.47 g cm^{-3}. Mineral segregation in density-gradient centrifugation is good, but not perfect because of the incomplete dispersion of minerals in the non-aqueous density gradient[10]. Sufficient quantities of either interleaved micas or muscovite micas may have been present in the light density kaolinite band to provide

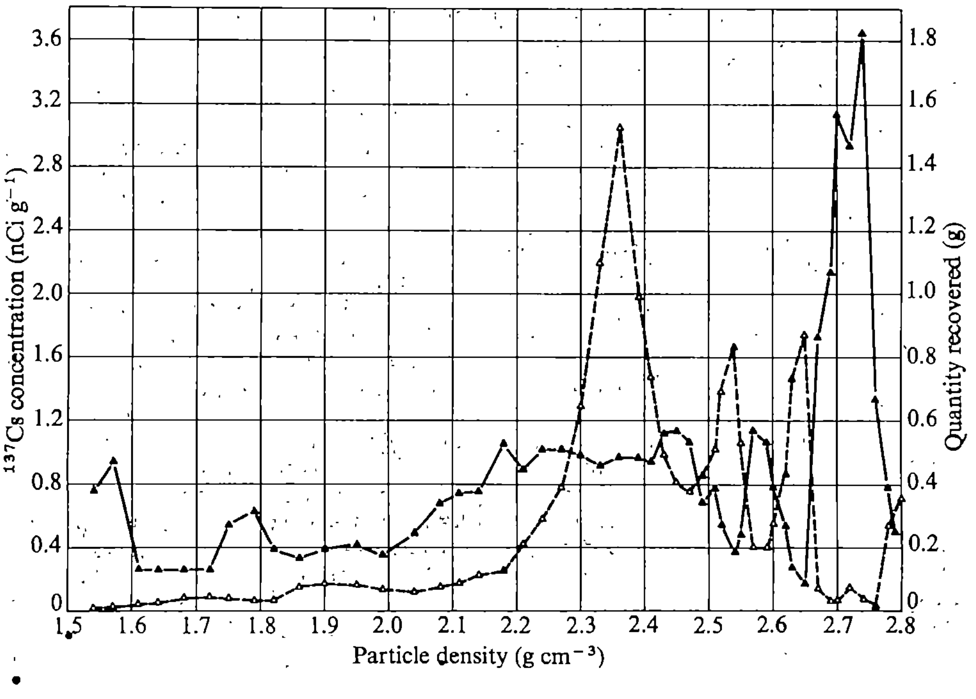

Fig. 1 Distribution of ^{137}Cs in sediment from Steel Creek, South Carolina. The concentration of ^{137}Cs in nCi g^{-1} (▲) and quantity of sediment recovered in g (△) at various densities (g cm^{-3}) after density-gradient centrifugation in a large scale zonal rotor.

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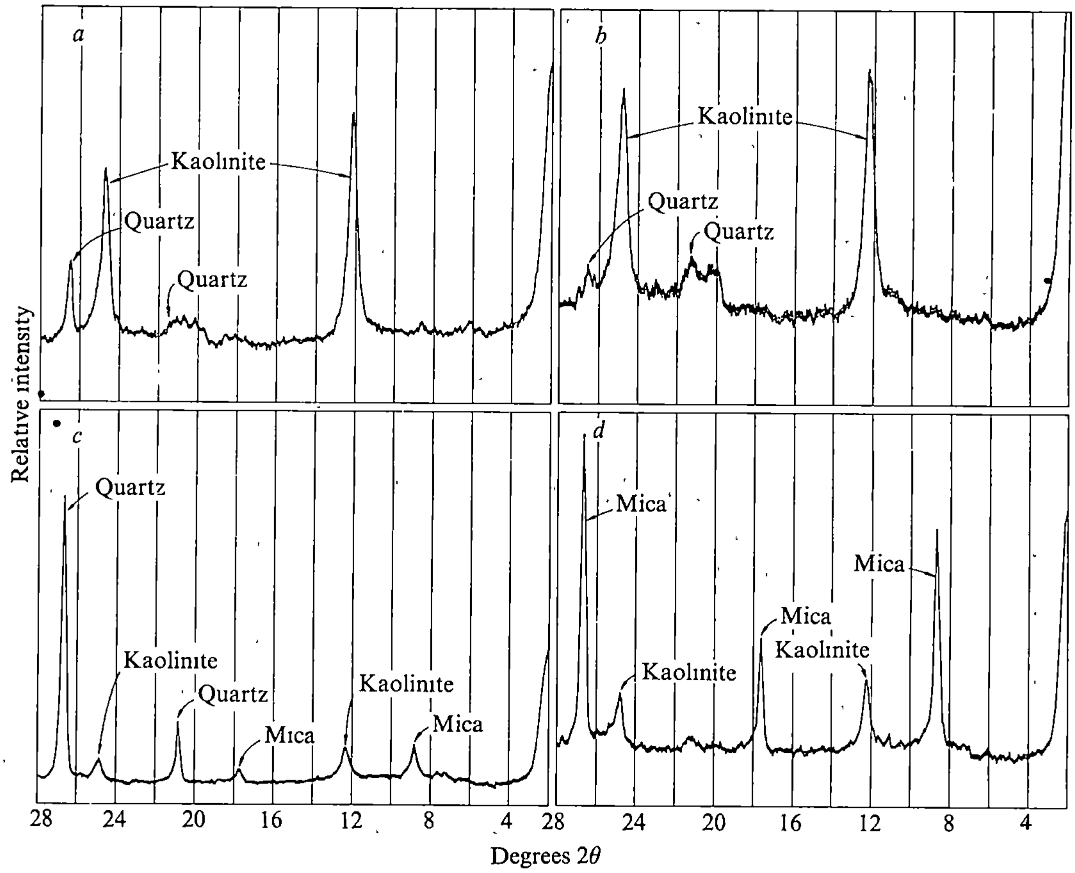

Fig. 2 X-ray diffraction patterns of the basal (001) diffraction maxima from oriented-aggregate mounts of *a*, Steel Creek sediment, <50 μm diameter; *b*, particle density fraction at 2.36 g cm^{-3}; *c*, particle density fraction at 2.65 g cm^{-3}; and *d*, particle density fraction at 2.74 g cm^{-3}. The abscissa units are degrees 2θ of Bragg's law and the ordinate reflects the relative intensities of the diffraction maxima using a Philips X-ray diffraction unit with a copper tube and nickel filter

'fixation' sites. For example, the ^{137}Cs associated with the density band identified as predominately a kaolinite mineral, is adsorbed to the weathered and unweathered mica minerals which cannot be detected by conventional X-ray diffraction procedures. Detailed examinations of a Georgia kaolinite using high resolution electron microscopy of ultramicrotomed sections of the kaolinite mineral revealed not only the expected 7 Å (001) spacing, but also occasional 10 and 14 Å spacings[12]. These higher interlayer spacings are thought to represent occluded and interleaved micas which will fix ^{137}Cs.

The consequence of selective adsorption of Cs to 'Cs-fixing' minerals in environmental conditions strongly influences the availability of ^{137}Cs cycling to the biotic component of a particular ecosystem. For example, as the ^{137}Cs diffuses from the less selective sites of soils or sediments to the more selective sites that 'fix' ^{137}Cs, the burden to biological organisms in that environment will be appreciably decreased. A case in point is the work of Eyman and Kitchings[13] who demonstrated that uptake of ^{137}Cs in bluegill (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*) from ingested clays containing adsorbed ^{137}Cs was ~ 8 times greater from montmorillonite and kaolinite than the interleaved mica, illite.

This work was supported by USERDA under contract with the Union Carbide Corporation and by the NSF-RANN Environmental Aspects of Trace Contaminants Program.

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Received October 6, 1975, accepted January 2, 1976.

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Oxygen isotopic evidence for the development of the psychrosphere 38 Myr ago

THREE major elements were involved in the evolution of oceanic circulation during the Cainozoic. The first of these was the diminishing role of oceanic meridional circulation in equatorial areas, the second was the development of circum-Antarctic circulation and the third involves the development of the present-day system of bottom waters of the world ocean, the 'psychrosphere'[1,2]. Because deep bottom waters are mostly derived from the polar regions, their characteristics, such as temperature, reflect surface conditions at high latitudes[3]. Past temperature changes of deep oceanic waters, and thus of polar surface waters, can be determined from the oxygen isotopic composition of deep-dwelling benthonic microfossils[3,4]. A deep-sea palaeotemperature record has been established[4] for the past 54 Myr based on DSDP sites in the sub-Antarctic; detailed arguments[4] give evidence that the Antarctic ice sheet developed during the Middle Miocene. Previous to this, however, a significant 5 °C decline in bottom-water temperature is recorded close to the Eocene–Oligocene boundary and is considered[4] to represent the onset of Antarctic bottom-water formation at temperatures close to freezing. This heralds the beginning of the modern thermo-haline circulation and is thus one of the most important palaeo-oceanographic events in the Cainozoic. We examine here

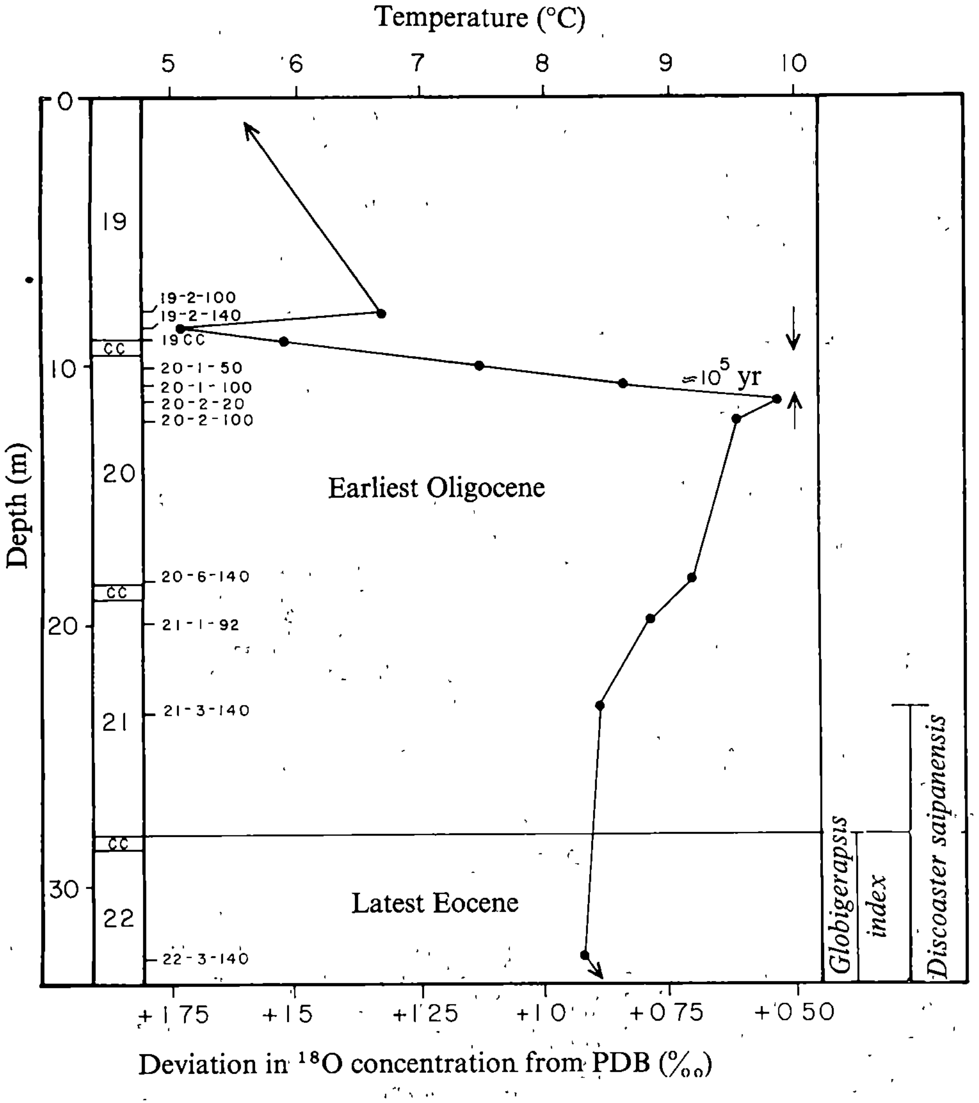

Fig. 1 Palaeotemperature curve and oxygen isotope data for benthonic foraminifera in latest Eocene–earliest Oligocene sediments of DSDP Site 277 (sub-Antarctic). Bottom temperature values were calculated using the procedure of Shackleton and Kennett[4]. The duration of the temperature drop of ~4 °C within core 20 is calculated from sedimentation rates at between only 75,000 and 100,000 yr. The Eocene–Oligocene boundary in this site is marked by the extinction of *Globigerapsis index*[6] as in New Zealand[32]. Thickness is plotted from top of core 19 which is 168.5 m below the ocean floor. Sample levels are shown.

in more detail the palaeotemperature event near the Eocene–Oligocene boundary, the latter having an age of 38 Myr (ref. 5).

Oxygen isotope measurements have been made on a series of latest Eocene to earliest Oligocene samples from cores 19 to 22 in DSDP Site 277, located on the Campbell Plateau in present day sub-Antarctic waters south of New Zealand (52° 13.43′ S; 166°11.48′ E; 1,222 m). This sequence consists of continuously cored late Palaeocene to late Oligocene foraminiferal-rich calcareous sediments[6]. About 38 Myr ago the southern part of the Campbell Plateau was at a palaeolatitude of ~ 65°S, 1,200 km nearer to Antarctica than now[7]. Samples of mixed benthonic foraminifera were analysed in 11 samples which consisted of ~ 0.1 and 0.4 mg carbonate after cleaning. Analytical methods, calibration and reproducibility of results have been discussed elsewhere[4–8]. The Antarctic continent was essentially ice-free before the Middle Miocene[4], and isotopic changes near the Eocene–Oligocene boundary are considered to have resulted from temperature changes.

A rapid reduction in bottom-water temperature from 10 °C to 5 °C during the earliest Oligocene is shown from the upper part of core 20 to the lower part of core 19 (Fig. 1), immediately followed by a brief warm oscillation (sample 19–2–100). Over half of the rapid temperature drop is represented in the uppermost part of core 20, the remainder between the upper sample of core 20 and the base of core 19 (sample 19–2–140). The sequence in core 20 represents a total recovery of 9 m (ref. 6) and thus the stratigraphic position of individual samples within this core is well established. The exact stratigraphic position of samples in core 19 relative to core 20 is unknown because of incomplete core recovery. Although there are now no geochronological methods to date the sediment of this apparently continuous sequence directly, the speed of the palaeotemperature change can be estimated using sedimentation rates. The rapid palaeotemperature change (Fig. 1) occurs over a thickness of only 170 cm. Sedimentation rates of ~1.6–2.2 cm per 1,000 yr for this interval[6] show that the duration of the palaeotemperature change was in the order of only 75,000–100,000 yr. About half (2.4 °C) of the temperature change recorded in the unbroken sequence at the top of core 20, took place in a period of 55,000–75,000 yr. The change occurs within the earliest Oligocene–latest Eocene *Globigerina brevis* foraminiferal zone[9] and the lower part of the *Reticulofenestra oamaruensis* to the top of the *R. placomorpha* calcareous nannofossil zones[10]. As in New Zealand, the Eocene–Oligocene boundary is placed at the extinction of *Globigerapsis index* (Fig. 1), and thus the temperature change occurs within the earliest Oligocene as recognised in temperate areas. At Site 277 the extinction of *Discoaster saipanensis* also precedes the isotopic event (Fig. 1). Further to the north in New Zealand, the extinction of *D. saipanensis* slightly precedes that of *G. index*[10]. A striking isotopic change in shallow-water benthonic fossils has also been shown close to the Eocene–Oligocene boundary in New Zealand sequences[11]. In the tropics, a decrease in bottom-water temperatures of ~5 °C is reported within the late Eocene between zones P15 and P17 (ref. 12), a change which must be isochronous with the sub-Antarctic event. This indicates that biostratigraphic correlations need modification between tropical, temperate and sub-Antarctic sequences. The extinctions of critical species probably occurred earlier in high latitude sequences creating diachronous usage of the Eocene–Oligocene boundary.

Our interpretation of the latest Eocene–earliest Oligocene oxygen isotopic change[4,11–13] is that it reflects a decrease in Antarctic surface-water temperatures to freezing and the production of extensive sea ice. This also marks the development of the psychrosphere. The oxygen isotopic evidence[4] indicates that no significant ice sheet formed at this time, and that the ice volumes on Antarctica remained relatively small until the Middle Miocene[4]. Ice-rafted debris in cores of Eocene and Oligocene age from the Southern Ocean off West Antarctica[14] record limited glaciation at this time, probably associated with alpine glacial development of West Antarctica[15], although others have suggested the presence of substantial thicknesses of ice since the beginning of the Oligocene[16]. In East Antarctica, ice conditions in the Palaeogene are unknown, but if glaciers were present, they did not reach sea level[17,18].

The drop in bottom-water temperatures in the earliest Oligocene caused a major crisis for the bathyal to abyssal benthonic fauna of the World's oceans including ostracods[2] and benthonic foraminifera[19]. The impact on planktonic fauna and flora was less dramatic but a simultaneous decrease (beginning in the late Eocene[20]) in surface water temperatures[4,11–13] caused a major reduction in diversity in planktonic organisms and a change in shallow-water echinoid fauna of South Australia[21] although these proceeded much less rapidly than in the deep-sea regions. A low diversity planktonic realm resulting from this crisis continued to the late Oligocene after which an increase in temperature resulted in increased diversity[22]. The planktonic microfossils at site 277 show no dramatic changes associated with the cooling[9,10].

It is now well established that the frequency of breaks or gaps in the stratigraphic record in all ocean basins reaches a maximum close to the Eocene–Oligocene transition[23–25]. There are also strong indications that the erosional event that created this disconformity commenced close to the Eocene–Oligocene boundary[7,24] and therefore is almost certainly linked with the development of Antarctic bottom water near the Eocene–Oligocene boundary. It is inferred that increased bottom-water activity caused erosion of deep-sea sediments in some areas and increased calcium carbonate dissolution in others, leading to mass wasting of calcareous sediments. The presence of widespread older disconformities[23,25] shows, however, that deep-sea erosion is not uniquely tied to the production of cold Antarctic bottom water.

Increased oceanic turnover, inferred to have commenced in the earliest Oligocene, is reflected by increased calcareous biogenic productivity in the central Pacific causing an increase in accumulation rates[26,27]. A major and apparently rapid deepening also occurred at this time in the calcium carbonate dissolution boundary creating a sharp contrast between carbonate-poor late Eocene and carbonate-rich early Oligocene deep-sea sediments[26,28,29]. Major palaeoenvironmental changes even occurred in shallow marine sequences related to extremely widespread shallowing of facies[30], although the causes of this sea-level lowering are unknown.

It is not clear what caused the rapid cooling of Antarctic waters 38 Myr ago. The Cainozoic palaeoclimatic development of Antarctica is marked by rather rapid temperature decreases superimposed on a steady climatic cooling after the early Eocene[4]. The Eocene–Oligocene boundary event therefore probably represents a critical threshold level in Antarctic climatic development, which in itself is related to more gradual isolation of the continent, resulting from the northward spreading of Australia and the opening of the Drake Passage. Intense circum-Antarctic flow did not develop until much later in the late Oligocene when the South Tasman rise split from Antarctica[7,31]. The timing of the opening of the Drake Passage is unknown, but is possibly linked to the palaeoglacial event 38 Myr ago[21,28]. Without additional Antarctic deep-sea drilling, the basic causal mechanisms of such Antarctic climatic changes will remain unanswered. In the meantime the events that occurred near the Eocene–Oligocene boundary will remain among the most profound for the entire Cainozoic.

This research was supported by the Office of Polar Programs, US National Science Foundation and NERC. We thank A. R. Edwards for useful comments on the manuscript.

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Grenville age for rocks in the Moine of north-western Scotland

THERE is considerable debate concerning both the age of sedimentation of the Moine Series and its tectonometamorphic history. Though several workers have argued for the presence of Precambrian metamorphic and tectonic features within the Moine (see, for example, ref. 1), others have considered these features to be essentially of Caledonian age (see ref. 2). In consequence, syntheses of the evolution of north-western Scotland during Proterozoic and early Phanerozoic time include the Moine either as an older part of the Dalradian sedimentary prism which became involved in both early and late Caledonian orogenic activity[3], or as a pre-Dalradian sediment wedge affected by pre-Dalradian (Morarian) subduction[4]

In spite of the growing number of mineral ages from several parts of the Moine outcrop which lie between 600 and 820 Myr (see, for example, ref. 1) and a "preliminary isochron indicating" a Rb–Sr whole rock age of 810 Myr (ref 5) for the Ardgour Gneiss, there is no firm, published,

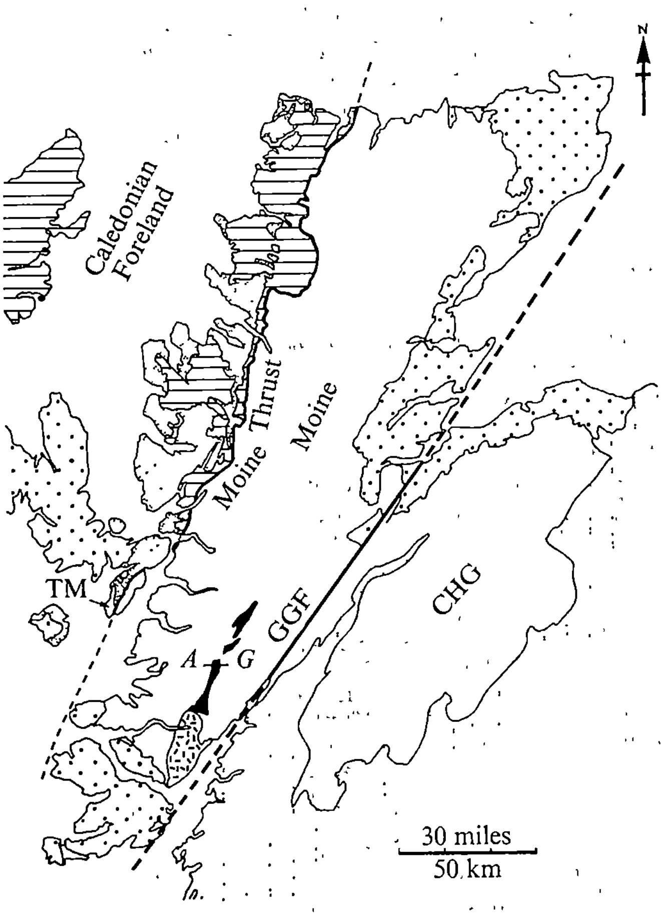

Fig. 1 Geological setting of the Ardgour Gneiss. Black, Ardgour Gneiss; *A–G*, location of sampling traverse; GGF, Great Glen Fault; coarse dots, cover and younger rocks; dotted lines, Dalradian; fine dots, Torridonian; horizontal lines, mainly Lewisian; CHG, Central Highland Granulites; TM, Tarskavaig Moine.

isotopic evidence for either the age of sedimentation of the Moine or the antiquity of subsequent orogenic activity.

Data are presented here for a Rb–Sr whole rock isochron for the Ardgour Gneiss at Glenfinnan (Fig. 1), which suggest strongly that the sedimentation of the Moine and the early thermal events which affect them, are of Grenville age[6].

The Ardgour Gneiss is a large body of migmatitic gneiss which has been considered as a metasomatic derivative of Moine metasediments[7–9]. The suggestion that it is a deformed and metamorphosed pre-orogenic granite has not gained support[8–10]. The gneiss has a generally concordant and transitional contact with surrounding, high grade, migmatitic Moine metasediments[7,8] and it occupies the core of a major second phase fold[8]. Neither Harry[7] nor Dalziel and Johnson[8] consider the gneiss to be diapiric but rather the result of partial fusion and metasomatism *in situ*. Structural elements contained by the gneiss indicate that metasomatism (migmatisation) took place after the first regional deformation of the Moine but before the second[8,10]. The migmatisation is thus coeval with the acme of the early regional metamorphism which preceded and overlapped these second phase movements[8,10].

Eleven whole rock samples of the Ardgour Gneiss were collected along a 1.5-km road section east of Glenfinnan (Fig. 1). Seven of these weighed between 16 and 30 kg, the remaining four between 2 and 3 kg. The Rb and Sr concentrations were determined using X-ray fluorescence spectrometry; ^{87}Sr/^{86}Sr ratios by mass spectrometry.

The data obtained (Table 1) define an isochron (Fig. 2) giving an age of 1,050±46 Myr and an initial ^{87}Sr/^{86}Sr ratio of 0.708±0.002. This is the age of the last strontium isotope homogenisation, which we consider occurred during the migmatisation. Following the methods of Hurley *et al.*[11], and Faure and Hurley[12], it can be calculated that the parent material of the Ardgour Gneiss would have had a mantle ^{87}Sr/^{86}Sr ratio of 0.700, 1,250 Myr ago. On this basis Moine sedimentation must have occurred 1,250–1,050 Myr BP.

If the conclusions concerning the derivation of the Ardgour Gneiss relative to the tectonometamorphic history of the Moine are correct it follows that the main, early, metamorphism took place about 1,050 Myr BP. Because it is associated with the second phase of deformation of the Moine[13] both the first and second major tectonic events affecting these south-western, or Morar, Moine are not only of Precambrian age but are broadly equivalent to similar events in the Grenville Province of Ontario[14] and the Gothides of Southern Norway[15]. If the age of sedimentation of the Stoer Group[16] of the Torridonian can be considered as 995±24 Myr (ref. 17) it is likely that Moine sedimentation was earlier.

It is significant that there is, as yet, no evidence for pre-Dalradian tectonothermal events affecting the Central Highland Granulites[18], supposed equivalents of the Moine lying to the south-east of the Great Glen Fault (Fig. 1). It seems that the Central Highland Granulites are much younger than the Moine *sensu stricto*[13] and are possibly equivalent to the Torridon Group[16] of the north-western forelands of the Caledonian Belt; perhaps to the Tarskavaig Moine[19] of the Kishorn Nappe; and to the Moine-like rocks of northern Mayo[20]. On the other hand, the Moine *sensu stricto*, may be equivalent to rocks of similar aspect in Ireland, namely those of the Ox Mountains[21] and Lough Derg[22], for which a Grenville age has already been suggested[21].

The implications in terms of crustal movements in north-western Scotland during the Proterozoic are that the Moine

Table 1 Analytical data for whole rock samples of the Ardgour Gneiss

Sample no.	Sample weight (kg)	Rb (p.p.m.)†	Sr (p.p.m.)†	^{87}Rb/^{86}Sr‡	^{87}Sr/^{86}Sr‡
Mo 42 (1)*	22.6	181	123	4.28±0.5%	0.7704±0.1%
Mo 43 (2)	26.5	176	147	3.48±0.4%	0.7581±0.1%
Mo 46 (3)	25.0	167	202	2.40±0.5%	0.7441±0.1%
Mo 46a (4)	2.20	171	204	2.44±0.6%	0.7441±0.1%
Mo 46b (5)	2.76	165	219	2.18±0.4%	0.7399±0.1%
Mo 46c (6)	3.17	156	239	1.90±0.3%	0.7359±0.1%
Mo 46d (7)	2.49	166	219	2.20±0.3%	0.7400±0.1%
Mo 47 (8)	17.6	166	136	3.57±1.7%	0.7617±0.1%
Mo 48 (9)	22.2	174	147	3.44±0.5%	0.7583±0.1%
Mo 49 (10)	17.1	172	170	2.94±0.7%	0.7517±0.1%
Mo 50 (11)	16.5	164	138	3.47±0.6%	0.7610±0.1%

*Numbers in brackets show number on Fig. 2.

†Approximate value.

‡Analytical errors derived from replicate XRF analyses and the overall reproducibility of ^{87}Sr/^{86}Sr measurements. These were used in the calculation of the regression line[23], which has a value of 1.1 for $S/(N\text{-}2)$ (ref. 24), fulfilling the criteria for an isochron.

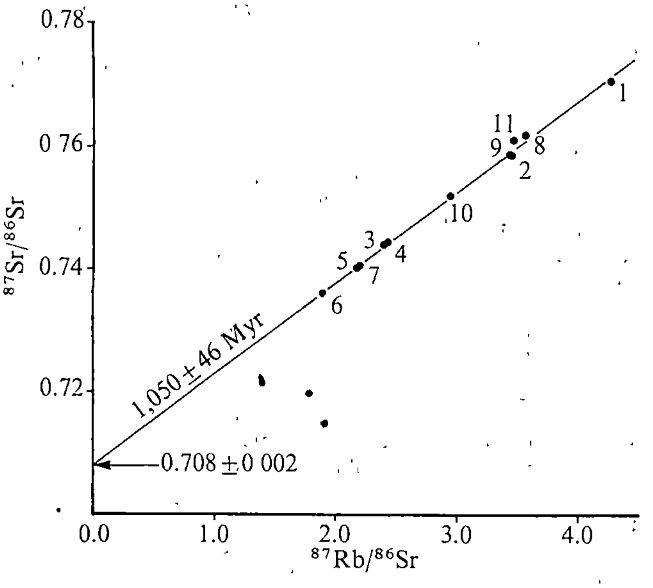

Fig. 2 Whole rock Rb–Sr isochron, errors quoted at the 2σ level. The ^{87}Rb decay constant is taken as 1.39×10^{-11} yr^{-1}.

achieved its orogenic condition some 600 Myr before the acme of subsequent Caledonian activity. Thus consideration of the Moine and Torridonian as broadly time equivalent sedimentary prisms on the western margin of the precursor to the Atlantic Ocean[3] is no longer tenable. The Stoer and Torridon Groups may have been deposited on a separate continental block from the Moine[4], but equally the Stoer Group could be considered as molasse derived during the Morarian orogen.

We thank N. J. Snelling for encouragement and advice, and J. Mitchell for help with sample preparation.

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Pre-Palaeozoic basement of the Scottish Midland Valley

THE tuffs and agglomerates associated with Carboniferous volcanism within the Scottish Midland Valley comprise predominantly basic volcanic and unmetamorphosed sedimentary fragments. For the most part, the former can be confidently ascribed to the contemporary basaltic volcanism whereas the sedimentary materials seem to be derived from Carboniferous and possibly Devonian strata. In spite of the fact that the bulk of the clasts within the volcanic vents are undoubtedly of shallow crustal origin and are unlikely to have had a vertical travel of more than a few thousand metres, others—in particular the spinel lherzolite clasts known from several vents—have probably arisen from subcrustal levels. In the Elie Ness vent, high pressure pyroxenes and pyrope garnets indicate rapid elevation from depths in excess of 60 km (ref. 1). It would be surprising if such violent, gas-fluidised eruptions, capable of carrying mantle materials, entirely failed to sample lower levels of the crust.

We have to call attention to a previously undescribed assemblage of metamorphic rocks at the Partan Craig vent [NT 567852] on the southern side of the Firth of Forth, some 1.5 km east of North Berwick Harbour. The locality is situated roughly midway between the lines of the Southern Upland Fault and the extended line of the Pentland Fault.

The clasts within and around the vent comprise mainly altered alkali basalt and basaltic tuff, with a wide variety of quartzitic sandstones and scarcer cherts, limestones and indurated shales. Some relatively coarse-grained biotite-rich mafic and ultramafic clasts also occur and include (altered) mica-peridotite and glimmerite. One clast seems to be an intensely carbonated (mica-poor) peridotite.

In addition, coarse-grained gneissic fragments may be found sparingly among the pyroclasts, the largest samples up to 1,500 cm in size. From a thin-section examination of 30 samples, two main categories may be distinguished. First, garnetiferous quartzofeldspathic gneisses; second, less siliceous (but still quartz-bearing) plagioclase-clinopyroxene gneisses. The larger blocks show a foliation with flattened quartz grains parallel to a model mineralogical layering, and the whole metamorphic assemblage may well have been derived from a coarsely banded basic and acid gneiss formation.

The feldspars range from sodic labradorite to oligoclase and, like the quartzes, display strain textures. In several clasts, string perthite is present. A preliminary examination of garnet from one clast indicates a composition approximately midway along the pyrope–almandine join. Kelyphitic rims to the garnets are ubiquitous. The pyroxene is a dull greenish augite ($\sim Mg_{37}Fe_{18}Ca_{45}$) which has been extensively replaced by carbonate and chlorite. Biotite, apatite, rutile and zircon are common accessories.

These features strongly suggest granulite facies metamorphism and recall the gneisses recently described by Strogen[2] from Carboniferous agglomerates in central Ireland. Though the age of these gneisses is as yet unknown the metamorphic grade is clearly higher than that seen in any of the Lower Palaeozoic rocks of southern and central Scotland, and the mineral assemblages are unlike those of the Dalradian rocks of the Grampian Highlands. This strongly suggests that the gneisses were brought up from a pre-Palaeozoic 'basement'. It is worth noting that, in spite of extensive search, no clasts were found in the vent that could be unequivocally assigned to the Lower Palaeozoic greywacke-shale successions of the Southern Uplands or Pentland inliers, the Lower Devonian basalt–andesite–trachyte–rhyolite succession of the Pentlands, the Dalradian metasedimentary–metavolcanic successions, or to any equivalents of the Ballantrae spilite–serpentinite–blueschist complex. Though too much weight must clearly not be given to such negative evidence, the possibility should be considered that at North Berwick, Upper Palaeozoic sediments directly overlie a gneissic basement complex The biotite-rich mafic and ultramafic clasts are probably genetically related to the basanitic magmas the degassing of which was responsible for the drilling of the vent.

The discovery of gneissic blocks at North Berwick lends support to the views expressed by Kennedy[3] and George[4] that the Dalradian Basin was bounded to the south by a Precambrian landmass underlying the Midland Valley and the region further south. In north-western Ireland, upper Dalradian (mid-Cambrian) turbidites could have been derived[5] from an uplifted basement of amphibolite and granulite facies lying to the south-east, of which the Deer Park Complex formed a part. Church and Gayer[6] also considered that some of the Connemara metamorphic rocks could represent exposures of a sialic basement to the Midland Valley structural unit. The concept of continental crustal rocks underlying the Southern Uplands and their Irish (Longford Down) continuation has both geological and geophysical support[2,7,8] and the new observations from Partan Craig would seem to confirm that a high-grade metamorphic complex also underlies at least a portion of the Midland Valley. It is difficult to reconcile the presence of these gneissic clasts with the various models proposed by Jeans[9], Gunn[10] and Mitchell and McKerrow[11], in which post-Arenig formations of the Midland Valley are postulated to overlie an earlier Palaeozoic oceanic crust.

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Cromagnon in the Iron Gate Gorge of the Danube

RADIOCARBON datings on human skeletons from the Padina site in the Iron Gate Gorge of the Danube in Yugoslavia have revealed that they belonged to a population at least 9,000 yr old This age is of especial importance because it is the earliest yet obtained by radiocarbon analysis for human bones from the region. It indicates for the first time the existence of a Cromagnon population in Yugoslavia.

During excavations at Padina[1], the remains of 37 individual skeletons, all in a very bad state of preservation, were uncovered. Anthropological study and laboratory examinations have shown that the skeletons possess all the morphological characters of the Cromagnon racial type. The skulls are long with huge supraorbital ridges and low vaults and the jaws are very large, with very rough areas for muscular attachments and with large prominent ridges and spines. The bones of the limbs are also large and robust with strongly marked muscular imprints. The average estimated height of the male individuals is approximately 180 cm (ref. 2).

Bone tissue for the analysis reported here came from the upper end of the best-preserved femora obtained. The specimens came from the lowest geological layer of the excavations. So far, two dates have been obtained· 6,487±83 yr b.c. (BM1144); and 7,248±103 yr b.c. (BM1147).

The Padina population lived in arc-like hollows beside the river bank which was intersected by rocky ridges and surrounded by wooded cliffs Alterations in the course of the river destroyed several such settlements, and gravely impaired the state of preservation of the human remains.

The excavations are into early Holocene deposits (~10000–6000 BC). At that time the climate, flora and fauna, and the general terrain and geography of Yugoslavia, became similar to those of the present day. The permanent snow cover of the Dinaric mountains melted, and grassland—bringing with it wild wheat, oats, barley and rye—spread along the Danubian plain. Wild goats, pigs, cattle and deer inhabited the forest[3]. It is very probable that in this region some Pleistocene hunters and gatherers adapted themselves to the new climatic conditions and migrated along the Danube, which offered them a new source of food Fishing became as useful as gathering and hunting.

Archaeological finds and cultural remains at Padina (and other sites such as Hajdučka, Vodenica, Lepenski Vir and so on[1] have not previously provided suitable material for accurate dating, although a radiocarbon date of 4,950 yr b.c. has been obtained from charcoal from the floor of one of the houses at Lepenski Vir (British Museum Radiocarbon Laboratory). Recently, similar material from Vlasac (not very far from Padina), examined at the Radiocarbon Laboratory in Zagreb has given a date of 5,609±93 yr b.c. (ZG 267); until this report, that was the earliest estimated age for these populations.

It is expected that further excavations further down the River Danube in the near future will reveal more human material in a better state of preservation, thus enabling a more detailed study of this newly discovered Cromagnon population.

I am grateful to the British Museum Research Laboratory for performing the ^{14}C analysis, and to the Radiocarbon Committee for financial support. I also thank the Institute of Archaeology in Belgrade, Yugoslavia, for providing the financial support for the field work and anthropological study

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Received January 23, accepted February 26, 1976

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Effect of sewage sludge on mineralisation of organic carbon in soil

SOME 40% of the sludge produced at inland sewage works in England and Wales is applied to agricultural land[1]. In addition to the plant nutrients and organic matter they provide, such sludges also contain various quantities of heavy metals[2]. In some cases, repeated and very heavy dressings of sludge have led to accumulations of harmful elements that are toxic to plants[3]. There has been concern about their possible accumulation in agricultural soils receiving sludge[4]

Table 1 Sludge analysis

		Consolidated activated sludge (AS)	Secondary digested sludge (DS)	Vacuum filter cake (FC)
Solids*		2.6	11.1	23.6
Organic matter†		67.8§	62	62
Macronutrients†	N	4.80	2.63	3 39
	P	2.54	1.97	2.27
	K	1.41	0.25	0.24
Trace elements‡	Zn	2,652	4,225§	5,650
	Cu	758	730	990
	Ni	30§	60§	340

* % wet weight.
† % dry matter.
‡ p.p.m. dry matter.
§ Figure for similar but not identical sample.

since, as Tyler[5] suggested, microbial activity in soils is susceptible to enhanced levels of heavy metals. The state of soils receiving sludge is monitored by regular analyses[6], and the Advisory Services of the Ministry of Agriculture (ADAS) have recommended a combined upper limit[7] to the total load (pre-existing and applied) of Zn, Cu and Ni, above which crop plants may be affected. We have investigated whether the approved quantities of sludge have adverse effects on the microorganisms responsible, in aerobic conditions, for mineralising the organic carbon in the soil, and have shown that three contrasting sludges from the same works, when mixed with soil in amounts up to five times the recommended limit, had no adverse effect on carbon mineralisation.

The three sludges tested (Table 1) came from a treatment plant receiving mixed domestic and industrial effluent, and they have all been used agriculturally. A single potting soil was chosen for this investigation because its properties (loam, pH 6.7, organic matter 4.1%) placed it near the centre of the range of agricultural soils. It was air dried and sieved through a 2-mm mesh. Samples of soil were treated with quantities of sludge—roughly equivalent to multiples of the maximum amount likely to be applied to agricultural land in one year (Table 2, level I)—thoroughly mixed, and placed in 15-cm pots. Maximum applications of two of the sludges (activated and secondary digested sludge) were limited by their high liquid content. Table 2 describes the sludge applications in three ways: as the actual amount of sludge added per treatment (*A*); as the ratio of the combined load of Zn, Cu and Ni (calculated according to ADAS procedure) to the recommended maximum load (*B*); and as the amount of organic matter added, expressed as a percentage of total soil (*C*). It should be noted that the three highest rates initially increased the soil organic matter by 25–250%.

The pots were planted with barley and set up in an illuminated greenhouse (minimum temperature 10 °C) for nearly a year, during which three crops were grown to the five-leaf stage and harvested. The barley was watered regularly, and the drainings recycled. The soils were allowed to drain and to dry out between crops and after the last crop. At this time samples of soil were taken from each pot, air dried, and again sieved through a 2-mm mesh. Their pH in water then ranged from 6.0 to 6.4.

Duplicate aliquots (10 g) of each sample were mixed with 0.5 g of ground oat straw (< 1 mm, 0.68% total nitrogen and 40.1% organic carbon), brought to 50% saturation with water containing ammonium nitrate to provide 100 p.p.m. N in the dry soil, and incubated at 30 °C Carbon mineralised as CO_2 was determined by the barium peroxide method[8] and expressed as p.p.m. of air-dry soil.

After 76 d incubation the pH in water ranged from 5.7 to 6.3, and soluble nitrogen (NO_3^- and NH_4^+ extracted with 1 M KCl) from 93 to 151 p p.m. dry soil, neither being related to sludge treatments. Clearly, carbon mineralisation had not been retarded by low pH or lack of available nitrogen. Figure 1 presents the cumulative CO_2–C release after 14, 35, 50 and 76 d. The lack of a consistent pattern in the results from the 0–III treatments indicates the degree of intra-treatment error.

There are only small differences between sludges and heavier applications of sludge increase the quantities of CO_2 produced, presumably in part from the organic carbon they contain. Since a parallel comparison showed no significant difference between the CO_2 produced by equal applications (350 g per pot) of filter cake, one of which had been first ignited to destroy its organic matter, it seems unlikely that the effects of the higher applications can be explained as reduced microbial activity on increased organic matter. So it seems that the overall activity (as measured by CO_2 production) of the organisms responsible for the decomposition of natural and added organic matter is unaffected by any of the types and levels of sludge applied.

Our highest sludge additions raised the copper concen-

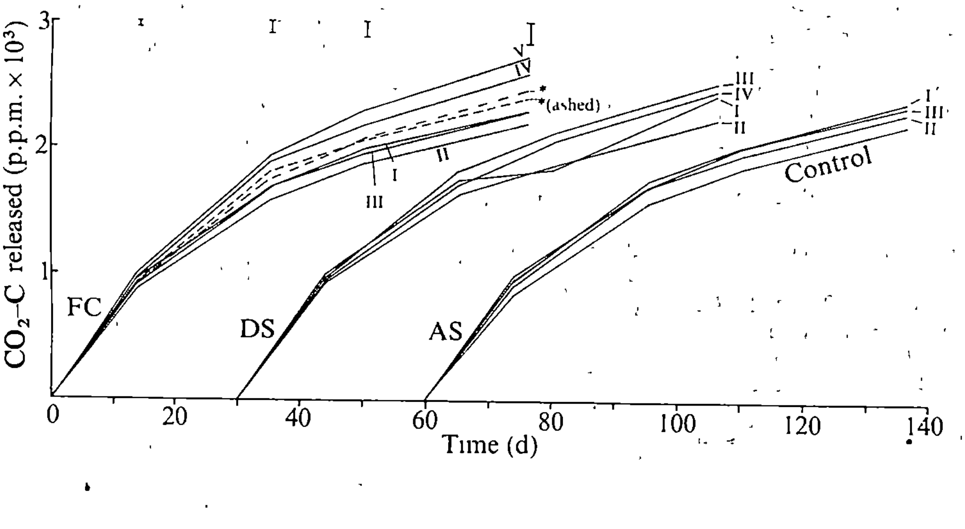

Fig. 1 Carbon mineralisation, measured as p.p.m. of C released as CO_2, in soil treated with increasing amounts (I–V, see Table 2) of consolidated activated sludge (AS), secondary digested sludge (DS) and filter cake (FC). Three control soils have been averaged. The pot treatments were not replicated and the least significant differences ($P < 0.05$) shown relate only to the two samples from each pot that were incubated separately. *Indicates a subsidiary comparison of ashed and unashed 350-g samples of FC.

Table 2 Sludge treatments

Level	A			B			C		
	AS	DS	FC	AS	DS	FC	AS	DS	FC
0	0	0	0	0	0	0	0	0	0
I	310*	75*	35†	0.08	0.11	0.18	0.29	0.3	0.3
II	630	150	70	0.16	0.22	0.36	0.6	0.56	0.55
III	1,060	300	140	0.27	0.44	0.72	1.0	1.12	1.11
IV	–	590	280	–	0.88	1.44	–	2.19	2.21
V	–	–	1,000	–	–	5.13	–	–	9.91

A, As the amount of sludge added per pot; *B*, as multiples of the ADAS maximum load of Zn, Cu and Ni, *C*, added organic matter as % of dry soil.
*ml.
†g fresh weight.

tration in the soil by 123 p.p.m., whereas Tyler[5] reported that the mineralisation of soil N may be adversely affected by copper concentrations greater than ~50 p.p.m. of dry soil. Nitrogen mineralisers are, however, thought to be more susceptible than carbon mineralisers, and Tyler's soils were very acidic (much more so than most agricultural soils in Britain). Heavy metals are widely reported to be more harmful in acidic conditions, so we do not find his or our results inconsistent.

We acknowledge financial support from the Thames Water Authority, and the assistance of Dr A. Milward, Mr V. H. Lewin and Mr D. L. Redhead.

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Human olfactory communication

IT has long been known that animals use their olfactory senses to communicate information, including sexual status, individual identification and maternal attraction[1-3]. Olfactory communication has been demonstrated throughout the Mammalia, including the primates[4,5], and there has been speculation as to whether or not it exists in some form in man[6,7]. Substances are know to exist on man that serve an olfactory function in other animals[8] and there are many apocrine and sebaceous glands on the human body that produce such secretions. We have done two experiments, the first to determine whether adults can identify an individual and determine his or her sex by the odour of an article of clothing, and the second to examine whether an infant can identify its mother's odour by a behavioural response

In the first experiment the subjects were 29 freshmen college students, 16 male and 13 female, all recruited by announcing the nature of the experiment in class and asking for volunteers The subjects were asked not to use any soap, perfume or deodorant for 24 h before the experiment and to wash only with clear water during this period. Then they were each given a plain white, appropriately sized, T shirt of 50% polyester and 50% cotton, and asked to wear the shirt as an undergarment for 24 h. The subjects were asked to don the shirts after class and remove them before class the next day. They were provided with new identical sealable plastic bags in which to place their shirts. The shirts were then collected, removed from the bags, and put in wax-coated cardboard ice buckets in which a one-inch triangular hole had been cut (to allow the subjects to sniff the contents). The ice buckets were then placed on waxed paper with the shirts arranged so that the under-arm portion was closest to the hole. Each subject was tested individually in a testing room. The placement of containers was randomised for each subject.

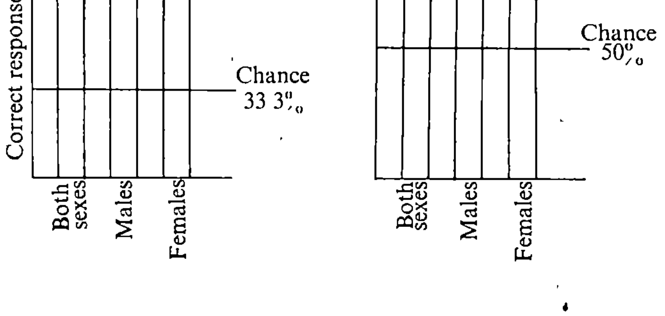
The first test was a three-choice discrimination consisting of: (1) the subject's own T shirt; (2) a strange male's T shirt, and (3) a strange female's T shirt. After each subject entered the testing room and was seated, he was asked to "sniff each bucket three times and indicate which one is yours—take as much time as you wish, and sniff as many additional times as you wish". Generally, each subject sniffed each bucket once in succession and then repeated this process.

The second test always followed the first and was the same except that it was a two-choice discrimination between the same strange male and the same strange female T shirt as used in the first test, and the subject was asked to discriminate the sex of the wearer by indicating which was male. Test 2 followed test 1, within a few minutes.

In each test, thirteen of the sixteen males and nine of the thirteen females answered correctly. This provided a highly significant result of $P=<0.001$ for test 1 and $P=<0.005$ for test 2 when a binomial expansion was used as a statistical measure.

While the number of correct responses was the same in each test, the individuals who answered correctly were not always the same. Several of the students remarked that they felt they would have been able to make the discrimination

Fig. 1 Experiment 1. *a*, Test 1 shows the percentage of correct responses in identifying subjects' own odour in a three-choice discrimination. *b*, Test 2 shows the percentage of correct responses in identifying male odour in a two-choice condition.



at a lower concentration of odour, but none of them mentioned finding either the strength or types of odour objectionable.

There was considerable variation in the concentration of the odour samples and no attempt was made to control diet, sexual cycles, or drug variations within the subjects or donors, yet most subjects could select the correct odour easily. During informal questioning after the test the male odours were usually characterised as musky and the female odours as sweet.

These tests show that at least the rudimentary communications of sexual discrimination and individual identification can be made on the basis of olfactory cues.

In the second experiment we investigated whether a similar type of ability could be demonstrated in an infant and, further, if olfactory discrimination would elicit a behavioural response. Several authors, including Darwin[9], have provided anecdotal reports that children may use odour to identify their mothers at an early age. Pratt *et al.*[10] reported that Pryer, Canestrini and Peterson Rainey in separate experiments obtained preliminary but conflicting evidence that infants may be attracted to the odour of their mothers' milk when nursing.

The subjects were recruited by asking mothers in the delivery ward of the University of California Moffitt Hospital to volunteer for the experiment. The subjects were 14 healthy full-term breast-feeding mothers (both multiparas and primiparas) and their infants. Tests were conducted on the second day, during the second week, and during the sixth week after birth. The olfactory stimulus used for these tests was obtained by asking each mother to place a breast pad (white cotton sponges) inside her bra for 3 h before testing. The mothers were also asked not to feed their babies during this period so that the infants would be somewhat hungry, but not distressed, during testing.

The test consisted of the experimenter holding a folded breast pad with thumb and index finger under the infant's nose at a distance of 1–2 cm for approximately 30 s while being careful not to touch the infant. During each test the infants were exposed to: (1) a clean moist pad; (2) a familiar (own) mother's pad, and (3) a strange mother's pad. For each infant the sequence of presentation was random. During the 6-week test the infants were also tested with a pad that had been moistened with raw cow's milk. Each test was conducted in either a standard hospital bassinet, or the infant's crib at home.

The test on day two was conducted in the hospital at the mother's bedside, and the 2- and 6-week tests were conducted in the home of the subject with the mother present. All breast pads were collected just before testing, except when it was necessary to travel between homes and then the pad from the strange mother was stored in a plastic bag The order of presentation of pads was random.

Infants were tested while sleeping whenever possible and not tested when distressed or crying. Pilot work had indicated that this was the only time that the infants could be tested independently of visual and auditory cues When an infant was aroused by the stimulus, time was allowed between presentations for the infant to return to its original behavioural state.

As Fig. 2 shows, during the rest on day two, only one of the 10 responded to any of the stimuli presented. That infant made a sucking response to both the strange and familiar odour.

At 2 weeks, eight of the 10 infants responded to the odour of the strange mother, seven responded to their own mother, and three infants showed a differential response between the two. A differential response occurred when the infant responded only to his/her own mother's pad and not to the strange mother's pad. In the case of the 2-week test nine infants responded, six similarly to both odours, two just to the strange odour, one just to the strange odour and one just to the familiar odour. At no time did any infant respond to the clean moist pad.

At 6 weeks only one of the infants responded to the strange mother, seven responded to the mother's pad and six showed a differential response. The most common response observed (in five of the infants) was a sucking orienting response to the mother's pad and no response at all to the strange mother's pad. A sixth infant who was hungry and sleeping when the test began did not respond at all to the presentation of the strange mother's pad, but awoke and began to cry when its own mother's pad was presented.

In each of the 6-week tests the responses to the raw cow's milk were the same as to the strange mother's pads.

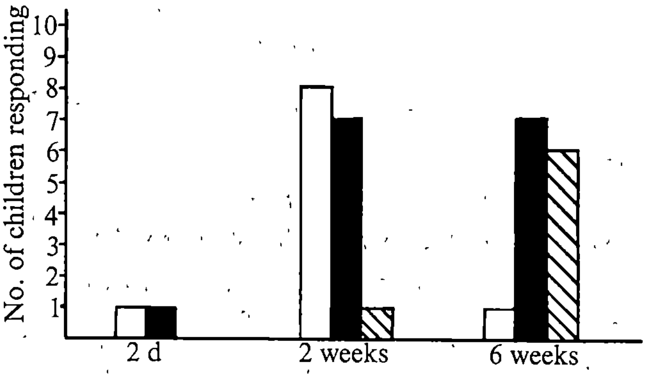


Fig. 2 Experiment 2. The responses of the 10 infants at 2 d, 2 weeks and 6 weeks to the strange mother's odour (open bars) and their own mother's odour (black bars). The hatched bar represents the number of children responding to their own mother and not to the strange mother.

Experiment two tested the infants' abilities to identify their mothers by odours left on breast pads at 2 d, 2 weeks, and 6 weeks after birth. At 2 d there was no response. At 2 weeks the infants showed general arousal and minimal, if any, discrimination. Then at 6 weeks six of the 10 infants tested could identify their own mother's odour from that of a strange mother's odour. At 6 weeks of age infants responded to their own mother's odours with a pattern of orienting and sucking that was markedly different from their response to both the strange mother's odour and the odour of raw cow's milk, which was added as an additional control. Furthermore, at least six of the infants showed a positive attraction to the mother and only one infant responded at all to the strange odour. This was a negative response, with a head jerk and a cry. The existence of olfactory maternal attraction suggests that humans have a pheromonal system and that it operates at a very early age. Since doing this work I have become aware of a strikingly similar experiment by Macfarlane[11] in which he was able to show a differential response at 6 d of age.

The initial identification of the mother may not be due to a response to her odours but rather to odours placed on her by the infant during earlier contacts, as demonstrated in other primates[12]. Furthermore, the possibility of this maternal scent marking in humans is supported by the common observation by parents of children who reject a favourite teddy bear or blanket after it has been washed because of the loss of odour acquired from earlier contacts.

Although the source of these odours has not been clearly demonstrated, olfactory cues seem to generate behavioural responses in infants, and sexual and individual identification seems to occur in adults.

This work was supported in part by a grant from Ms Imelda Finnigan and the National Institutes of Health. I thank Ms Margaret Rooney, Larry Elam, Dr Joseph Kitterman and Marc Russell for help

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Importance of antennae for orientation of insects in a non-uniform microwave electromagnetic field

MICROWAVES can affect living organisms in different ways[1]. Radiation of high intensity is lethal, due to the thermal effect on the living tissues. Different organs are selectively sensitive both in vertebrates[2] and in insects[3]. Behavioural studies with rats have demonstrated that, in a choice situation, the animals will respond to an electromagnetic field of a rather low intensity, by leaving it[4]. Our investigations revealed that insects respond to the intensity gradient of a non-uniform microwave electromagnetic field in a similar way. In an arena illuminated by a non-uniform field insects carry out oriented movements avoiding the regions which are more intensely illuminated. In this paper we analyse the involvement of antennae in the orientation mechanisms underlying the avoidance response.

Adults of *Caryedon gonagra* (Fab.) and last instar nymphs of *Pyrrhocoris apterus* L. were used. The former species represents a beetle with low tendency to crawl, the latter one an active and fast moving bug. Antennectomised and intact specimens were tested in a round arena constructed from a polystyrene Petri dish. The arena was placed on the broad wall (bottom) of the waveguide of rectangular cross section (Fig. 1*a*). In this position a symmetrically non-uniform electromagnetic field was obtained inside the arena, with maximum flux density (power density) occurring in the axial E plane of the waveguide. The insects were released in the centre of the arena and all individuals avoiding the most intensively illuminated central band of the arena were counted every 60 s for 10 consecutive min. The width of the band was arbitrarily chosen so as to enclose the region of power densities greater than 50% maximum power density (Fig. 1*b*). The observations were made at five different power densities and the controls observed at zero electromagnetic field intensity. Behaviour of the beetles and bugs was observed three and five times with 11 and 5 fresh specimens, respectively. The maximum numbers of individuals observed at each repetition outside the central band after cessation of response, were summed up; the figure obtained served to express the avoidance response at a given power density. The significance of differences was estimated on the basis of a χ^2 test. Mortality was expressed as percentage of dead animals at the end of the 10-min observation period. A TESLA 50SA51 magnetron was used to generate the microwave power. Incident power levels used ranged from 100 to 1,200 W in a continuous wave (CW) regime and at a frequency of 2,375 MHz. The maximum power density inside the dish for the transverse electric (TE_{01}) mode used was 3 3–40 W cm^{-2}.

At zero incident power the beetles *C. gonagra* (Fig. 1*c*) tend to remain near the site of release. At 3.3 W cm^{-2} both antennectomised and intact specimens retained a tendency to remain in the central band. When electromagnetic power of increasing intensity was applied the intact animals began to respond by moving out of the central band at power densities of 6.6 W cm^{-2}, the avoidance response being highly significant at power densities greater than 6.6 W cm^{-2}. The corresponding threshold value for significance of the response of antennectomised beetles was at power densities greater than 13.2 W cm^{-2}. Lethal effects of microwaves were observed only at highest intensities used and predominantly in the antennectomised individuals.

Both antennectomised and intact nymphs of *P. apterus* (Fig. 1*d*) spread more or less equally to the periphery of the arena at zero incident power and quickly changed their positions

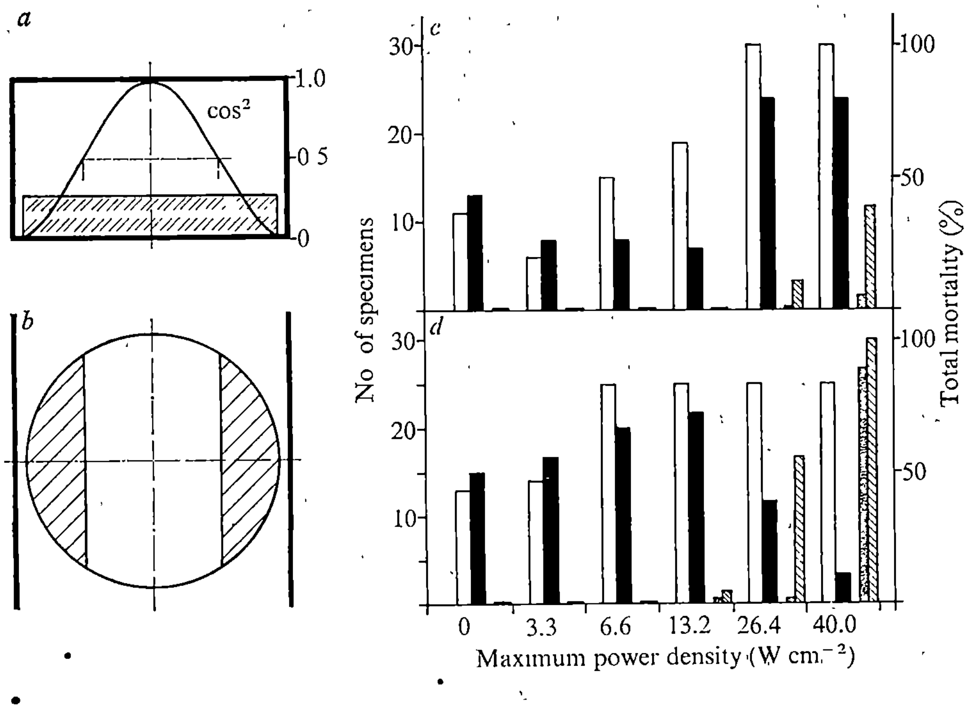

Fig. 1 *a*, Cross section of waveguide showing microwave energy distribution (cos^2) across arena. *b*, Surface view of arena; central (clear) band receives power densities greater than 50% maximum. Avoidance of central band by *C. gonagra* (*c*) and *P. apterus* (*d*) in intact (open columns) and antennectomised specimens (solid columns), and mortality in intact (stippled columns) and antennectomised specimens (hatched columns).

thereafter. Significant avoidance response was recorded only in intact specimens at power densities 6.6 W cm^{-2} or more. The response in antennectomised bugs was not statistically significant, although they showed a slight tendency to avoid the central band at higher power densities. Mortality was higher in the antennectomised specimens than in the intact ones.

These experiments thus provide two lines of evidence for importance of antennae in the orientation of insects along the intensity gradient of a microwave electromagnetic field. First, the threshold incident power levels for initiating the avoidance response are higher when the antennae are lacking. Second, the lack of antennae increases vulnerability of insects to microwave energy non-uniformly distributed over the arena, apparently because of impairment of their orientation mechanisms. A certain ability, although significantly reduced, of antennectomised individuals to avoid the field of intensive microwave radiation, however, suggests that still other mechanisms of orientation, which do not depend on sensory input from the antennae, may exist. We hesitate to speculate about their nature as well as about a possible biological significance of antennal sensitivity to microwaves. The threshold densities causing aversion in intact insects are 1,000 times higher than those in rats[4]; however, they are still well below the lethal dose (J.O., J.Ž. and J.D., unpublished). We suspect that in our experiments the aversion induced by microwaves is mainly due to thermal effects. Nevertheless, the sensitivity of intact specimens to rather low densities (6.6 W cm^{-2}) of the microwave field may indicate that the sensory apparatus of insects can detect, and that the animals can respond to, quantities of radiation producing no harmful effects. Nonthermal effects of microwave radiation on developing insect tissues have already been demonstrated[5].

We thank Dr B. Holubičková, Mathematical Centre of Biology, Czechoslovak Academy of Sciences, Prague, for assistance with the statistical evaluation of the results.

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Received October 20, 1975, accepted February 10, 1976.

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Non-ageing developmental variant of *Caenorhabditis elegans*

THE study of mechanisms affecting the rate of ageing can be facilitated by naturally occurring phenomena, innate to some organisms, that enable the species to retard its ageing rate and extend its life span[1]. Such a phenomenon exists in certain species of nematode. Larval forms of the free-living soil nematode, *Caenorhabditis elegans*, possess the ability to enter a semi-dormant, quiescent state referred to as the dauer larval stage (German, "enduring" larva). Newly hatched larvae of *C. elegans* undergo four larval stages (L1–L4) punctuated by moulting. If larvae are starved, they will enter the dauer state during the second larval moult. At this time, the old cuticle is replaced by a special, relatively impermeable cuticle unique to dauer larvae[2].

During this state the larvae cease to feed, the pharyngeal muscles become inactive and all development ceases. Dauer larvae are thermotactically opposite to non-dauer larvae[3] and are resistant to harsh chemicals such as 1% sodium dodecyl sulphate (SDS)[2]. Although no food is ingested, the dauer larvae are capable of moving during the dauer state. Dauer larvae maintain a high degree of motility close to or greater than that of non-dauer larvae for the first 2–3 weeks after dauer formation. This motility never completely ceases but declines gradually to a minimum by the end of the dauer life span (approximately 70 d). All dauer larvae used in these experiments were moving. Placing dauer larvae in a bacterial suspension of $\geq 5\times10^8$ bacteria per ml will stimulate the larvae to come out of the dauer state, undergo the third and fourth larval moults, and become mature, productive adults.

Fig. 1 *Caenorhabditis elegans* var. Bristol was cultured in liquid S-medium supplemented with *E. coli* strain OP50 at 10^9 bacteria per ml as described previously[4]. All cultures were grown at 20±1 °C. Initial inoculum was 6 worms per ml. 0, Time of hatching. △, Number of bacteria per ml as determined using Petroff Hauser counter; ○, worms per ml; ●, dauer per ml as determined by resistance to 1% SDS[2].

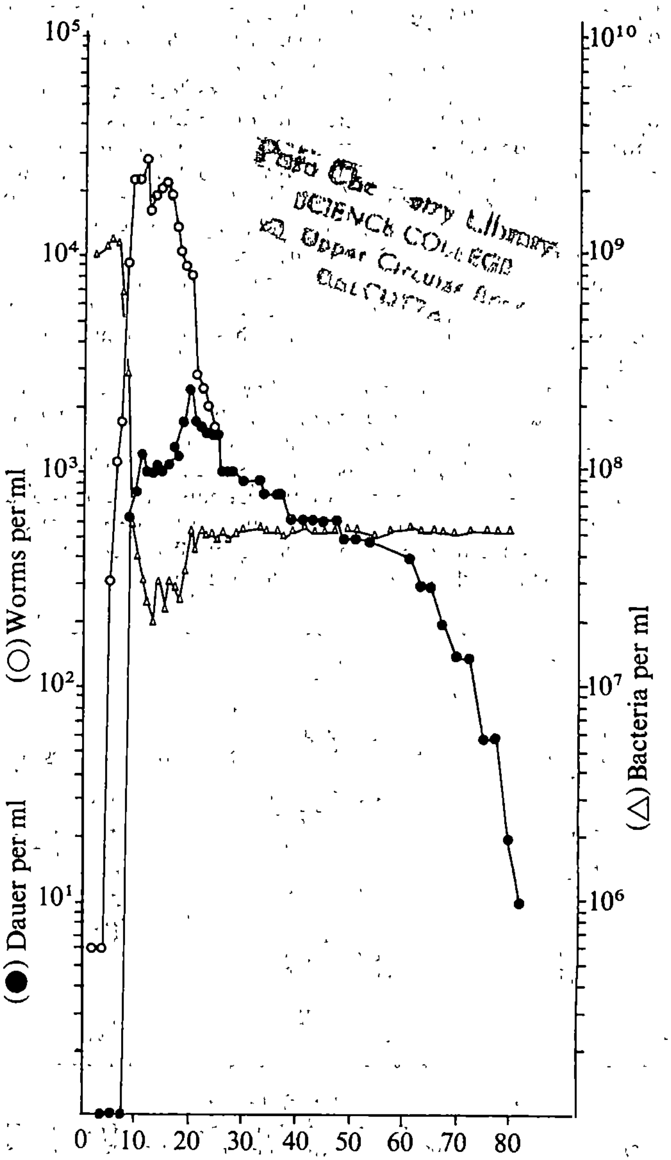

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Table 1 Effect of duration of dauer state on life span

Duration of dauer state (d)	Post-dauer life span (Mean ± 95% c.i.m.*)	Mean no. of eggs	Egg viability (% hatch)	Total life span
5	11.16±1.20	283	100.0	16.16
10	10.08±0.96	330	98.1	20.08
15	9.68±1.16	236	97.7	24.68
20	10.89±1.13	304	100.0	30.89
25	10.19±1.08	323	91.3	35.19
30	10.53±0.93	242	93.4	40.53
35	9.38±1.12	282	97.4	44.38
40	10.23±1.42	270	95.0	50.23
45	9.90±1.47	290	84.8	54.90
50	10.16±1.71	339	100.0	60.16
55	11.05±1.85	274	94.2	66.05
60	10.24±1.81	394	97.7	70.24

*c.i.m., Confidence interval of the mean.

Dauer larvae were isolated by the SDS resistance method of Cassada and Russell[2] and were maintained in M-9 salt solution[4] At 5-d intervals samples of 100 dauer larvae were removed and placed on NGM agar plates seeded with a drop of *E. coli* (10^9 ml^{-1})[4]. Percentage survival, number of eggs laid and egg viability were determined at daily intervals at 20 °C. The mean life span of nematodes that never entered the dauer state at 20±1 °C is 12.76±1.08 d (n=50) and the average number of fertilised eggs produced per parent is 280±40 (±95% c.i m.*; n=25). Normal egg viability is ≥97%.

Dauer larvae can be maintained for more than 70 d (Fig. 1). The 50% survival time is approximately 45 d at 20 °C. There is a small increase in the frequency of post-dauer mortality (Fig. 2). As the duration of the dauer state increases, the percentage of larvae which fail to recover from the dauer state increases from 0% at 10 d to 32% at 60 d. This effect is probably due to the reduced vitality brought on by starvation.

The duration of the dauer state has no effect on the post-dauer life span (Table 1). There is no observable effect of the dauer state on (1) post-dauer life span; (2) post-dauer egg production; or (3) egg viability. Worms that have been in the dauer state for 60 d have the same post-dauer life span, lay the same number of eggs, and have the same egg viability as worms that have been in the dauer state only 5 d.

Dauer larvae have a primordial gonad composed of approximately 35 nuclei. The duration of the dauer state has no effect on the life span of the F_1 progeny which are descendants from these germ cells (Table 2).

If ageing in *C. elegans* is a result of random accumulation of damage (nuclear or cytoplasmic) due primarily to external insult for example, ultraviolet, X rays, cosmic rays, and so on, then it is reasonable to presume that prolonging the dauer state would be correlated to an increase in accumulated damage and subsequently a decrease in the post-dauer life span. This result was not found. There was no detrimental effect on life span observed by extending the dauer state up to 60 d. Mean post-dauer life span remained constant and the total life span simply increased by the amount of time spent in the dauer state.

Table 2 Life span of progeny of experimental worms

Duration of dauer state (d)	F_1 Life span (Mean ± 95% c i.m.*)	Mean no of eggs
5	11.10±1.29	275
10	12.47±1.02	279
15	11.70±1.19	198
20	11.59±1.01	224
25	10.13±0.94	269
30	11.44±0.75	294
35	11.02±1.15	198
40	11.70±1.33	169
45	11.52±1.18	264
50	11.78±1.37	273
55	11.53±1.21	199
60	10.77±1.32	248

*c.i.m., Confidence interval of the mean.

Samples of 100 F_1 larvae were taken on the second day of egg laying from parents who had been in the dauer state for increasing durations. The life span of the F_1 larvae and the average number of fertilised eggs produced by the F_1 worms were measured.

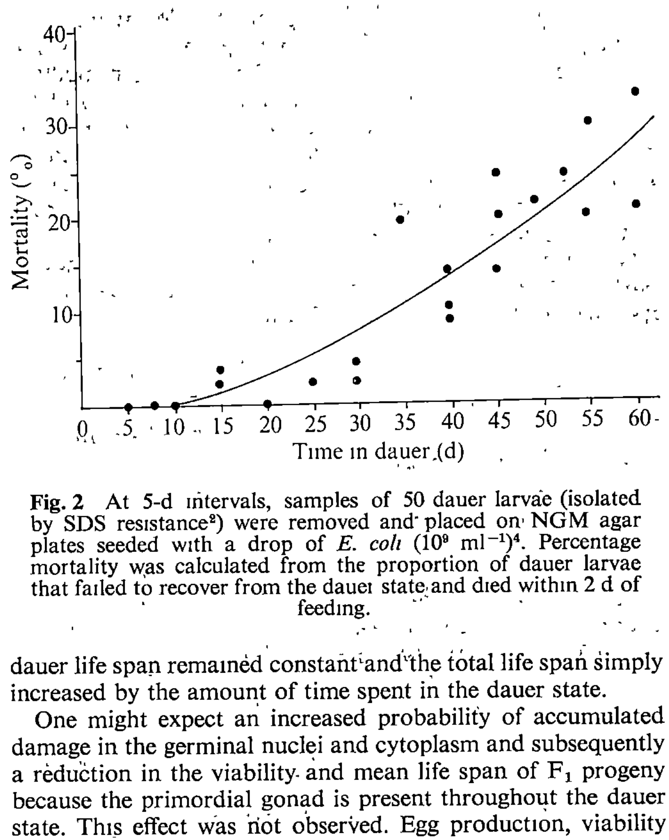

Fig. 2 At 5-d intervals, samples of 50 dauer larvae (isolated by SDS resistance[2]) were removed and placed on NGM agar plates seeded with a drop of *E. coli* (10^9 ml^{-1})[4]. Percentage mortality was calculated from the proportion of dauer larvae that failed to recover from the dauer state and died within 2 d of feeding.

One might expect an increased probability of accumulated damage in the germinal nuclei and cytoplasm and subsequently a reduction in the viability and mean life span of F_1 progeny because the primordial gonad is present throughout the dauer state. This effect was not observed. Egg production, viability and F_1 life span remained constant. It must be concluded that because there is no life-shortening effect of prolonging the dauer state up to 60 d (four times the normal life span) externally induced damage is not a primary cause of ageing in *C. elegans*. Normal ageing therefore must be an innate process whose rate is directly related to the metabolic rate.

The phenomenon of dauer larvae has been observed and described for many years. Many parasitic forms have infective larvae which can survive for extended periods in a dauer-like state. If they contact a host and subsequently develop, there is no correlation, above a survival threshold, between "age" of the larva and its ability to become an adult[5]. Our observations with *C. elegans* parallel the results obtained with parasitic forms.

Dauer larvae seem to be able to reduce or suspend the rate of ageing. This "non-ageing" quiescent state offers an interesting model for the study of mechanisms that control the ageing process. Analysis of the developmental processes that enable a metabolically active, rapidly growing larva of *C. elegans* to enter a developmentally dormant, "non-ageing" state may offer valuable insight into the controlling mechanisms of ageing.

This work was supported by a US Public Health Service grant from the National Institute on Aging.

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Received November 6, 1975; accepted February 3, 1976.

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Graphs of contracting glycerinated *Amoeba proteus*

GLYCERINATED *Chaos-Proteus* amoebae preserve their normal shape, but will contract after washing and introduction of physiological concentrations of ATP, Ca^{2+}, and Mg^{2+} (ref. 1). We have recorded the motion of activated glycerinated amoebae (amoeboid models) in a manner such that endoplasmic motions, shape changes, and contractile sites are pictorially graphed by the model during its contraction. Our findings contradict the frontal-zone contraction hypothesis, but do not unequivocally support the ectoplasmic tube contraction theory.

Glycerination of *Amoeba proteus* was carried out in 50% glycerol (v/v), EDTA 5 mM, Tris-HCl buffer 0.01 M, KCl 0.05 M, *p*H 7.0, at ~10 °C. It is critical to reduce the medium to a small volume containing a few starved amoebae and then rapidly to apply a large volume of this solution. Glycerination was continued for at least 24 h and its quality was judged by retention of amoeboid shape and by subsequent contractility of the model. Actual contractions of amoeboid models were in 25% glycerol (v/v) with ionic contents and buffers as above plus ATP, Ca^{2+}, and Mg^{2+}, each at 0.5 mM. The time for contraction varied from 1 to 10 min following ATP–Ca–Mg application.

Contractile dynamics were recorded under darkfield microscopy at low power (×60), and photographs were taken sequentially at 1-min exposures with ~5-s intervals. Figure 1 is a sequence of four photographs recording movements of amoeboid model contraction. The first photograph shows the amoeboid model washed immediately after adding ATP–Ca–Mg. Cytoplasmic granules appear white in the photographs and their horizontal displacement is recorded as streaks whose lengths vary with their velocities[2]. Granules that are moving vertically will show only a sharp single image. The beginning of contraction (second photograph) is indicated by the streaks of granules in motion. Their velocity can reach 50 μm min^{-1}. The third photograph in this sequence illustrates the continuing contractility of this model. The last photograph is the contracted amoeboid model, indicated by a lack of recorded motion.

Study of these photographs facilitates precise determination of the contractility sites and the resultant motion of this model. The outlines below the fourth photograph summarise the visible changes in shape displayed by this glycerinated model. Measurement of the large leading pseudopodium reveals a slight change in length, emphasising that contraction occurs predominantly in the width of the model. Ectoplasmic contraction towards the central portion would account for this observed motion.

Figure 2 is a three-photograph sequence of a glycerinated model illustrating that pressure can be generated by contraction, as indicated by endoplasmic displacement. In the first and second photographs, at pseudopodial junctions near the tail end (bottom of photograph), prominent streaks can be seen in the central portion of the model, indicating endoplasmic motion. The volume change during contraction of this model is relatively small. The last photograph of this sequence records the endoplasm being forced out of an ectoplasmic rupture into the environment.

These photographs of ATP–Ca–Mg-activated *A. proteus* models demonstrate that contraction first, is localised in the ectoplasmic tube; second, can displace glycerinated fluid-like endoplasm at a velocity reaching 50 μm min^{-1}; and third, can generate a pressure within these models displacing the fluid-like endoplasm.

The ectoplasmic tube contraction theory (ETC)[3] requires that ectoplasmic tube contraction produces a pressure which displaces relatively non-contractile endoplasm; these specific requirements are supported by this investigation. Further, during normal amoeboid motion, the ETC theory predicts some interconversion of ectoplasm to endoplasm at its interface as a part of the contraction process. Ectoplasm to endoplasm interconversion may occur once, but it is not a continuous feature during contraction of glycerinated models. Reversible relaxations and contractions of these models were not achieved. Amoeboid models can contract, but they do not exhibit organised amoeboid motion, which might be explained by the lack of ectoplasm to endoplasm interconversion.

Frontal endoplasmic contractions were never observed in our studies, which conflicts with the fundamental contractile requirements of the frontal-zone contraction hypothesis[4,5] for amoeboid motion.

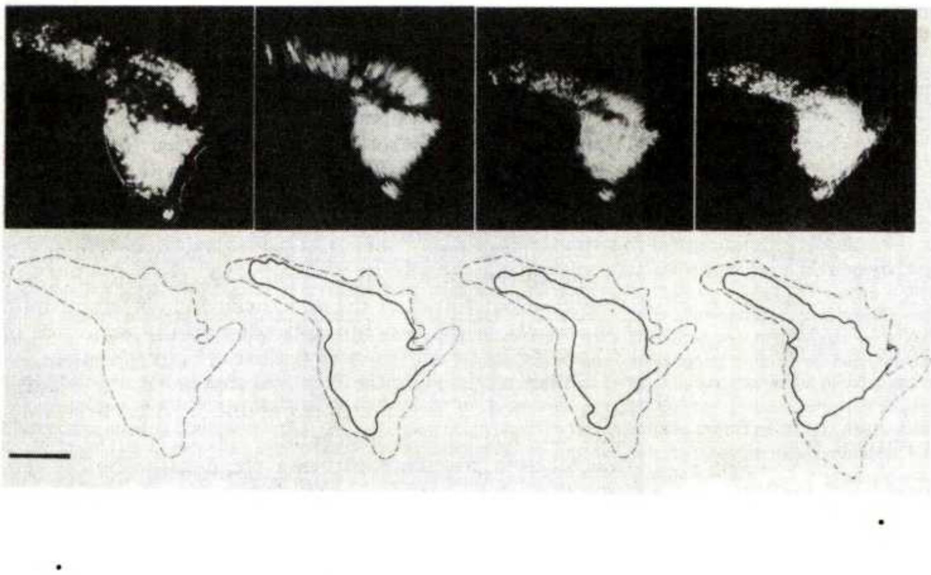

Fig. 1 Graphic sequence of a single glycerinated model contracting: - - - -, Before contraction; —, outline of contraction state of photograph directly above it. The bar represents 100 µm.

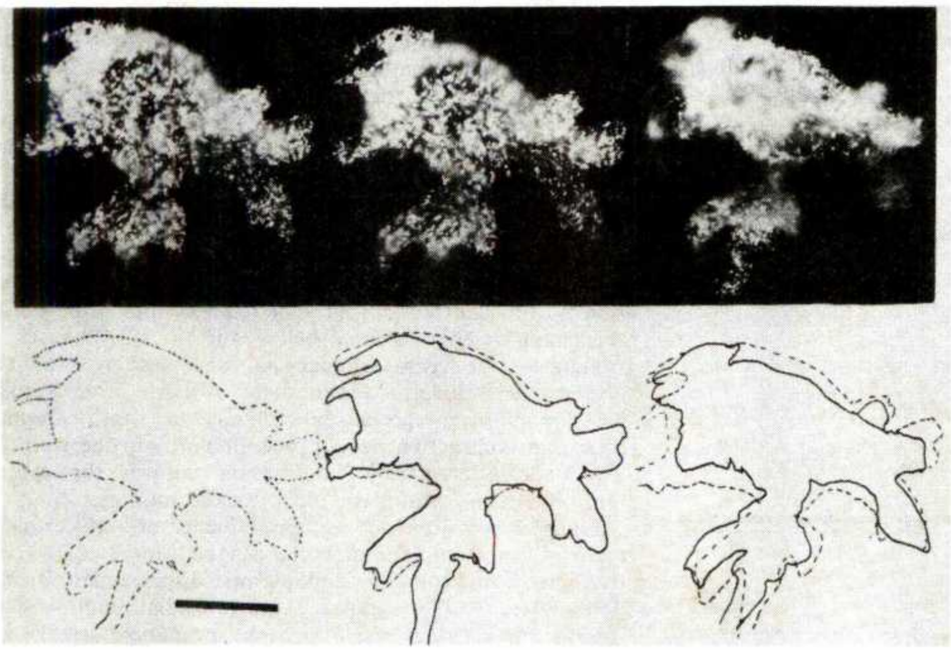

Fig. 2 Sequence illustrating amoeboid model contraction, displacement of endoplasm, rupture of plasmalemma (ectoplasm) and expulsion of some endoplasm. – – – –, Gives the beginning of contraction; —, is photograph above. The bar is 100 μm.

In preliminary experiments, the endoplasm of glycerinated models was moved reversibly in either direction by applied external pressures at values which have been found to move amoeboid endoplasm *in vivo*[6,8]. Pseudopodia could be collapsed or expanded by pressure depending on flow of the glycerinated endoplasm.

Numerous investigators have reported muscle-like proteins in amoeba (see refs 9–11). Some actin-like filaments may be attached to the plasmalemma[9]; whereas, myosin-like proteins do not always display a 'thick' filament component as reported in *Acanthamoeba castellanii*[10]. But thick filaments can have ATPase activity[10]. Also, thick and thin filaments have a remarkable orientation and interdigitation in the ectoplasmic tube region of *A. proteus* similar to striated muscle cells[11]. Although thick and thin filaments are present in glycinerated *Chaos-Proteus* amoebae, these filaments, their interactions and sites during contraction–relaxation have not been determined by electron microscopic investigation. It seems, however, that sliding filaments as proposed for muscle contraction[12] constitute the basic contractile mechanism for amoeboid motion[13,14].

We thank the Polish Academy of Sciences, Professor L. Kuznicki, the staff of the Cell Biology Department, and the M. Nencki Institute of Experimental Biology, Warsaw, Poland, for their help.

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Bilateral cortico-tectal projection from the visual cortex in the cat

THERE is anatomical and electrophysiological evidence for a heavy and well organised fibre projection from the visual cortex on the superior colliculus of the midbrain in several species[1–4]. In the cat, areas 17, 18 and 19 of the visual cortex each project to the colliculus and the arrangement is such that a point in the cortex related to a particular part of the retina sends fibres to that part of the colliculus receiving fibres directly from the same part of the retina. The available evidence is for a cortico-tectal projection to the ipsilateral superior colliculus only. In several brains of cats with lesions involving the visual cortex of one hemisphere, fibre degeneration has now also been found in the superior colliculus of the opposite side. This observation provides an explanation for several findings made in electrophysiological studies of the representation of the visual field in the superior colliculus which have been difficult to interpret, and it should help in the interpretation of functional studies on the visual connections of the cerebral hemisphere.

Studies of the visual system of the cat have made available a number of brains with lesions of varied size in the visual cortex and in the superior colliculus. The cats were anaesthetised with intraperitoneal Nembutal and, with aseptic precautions, lesions were made under direct vision in the visual cortex of one hemisphere or electrolytically in the superior colliculus of one side by a horizontal stereotaxic approach through the cerebellum. After survival periods of 4–6 d the animals were perfused with saline and 10% formalin and the brains were immersed for a further period in the same fixative. Frozen sections were cut in the coronal plane and a 1 : 10 series of sections stained with the Fink–Heimer or Wiitanen methods[5,6].

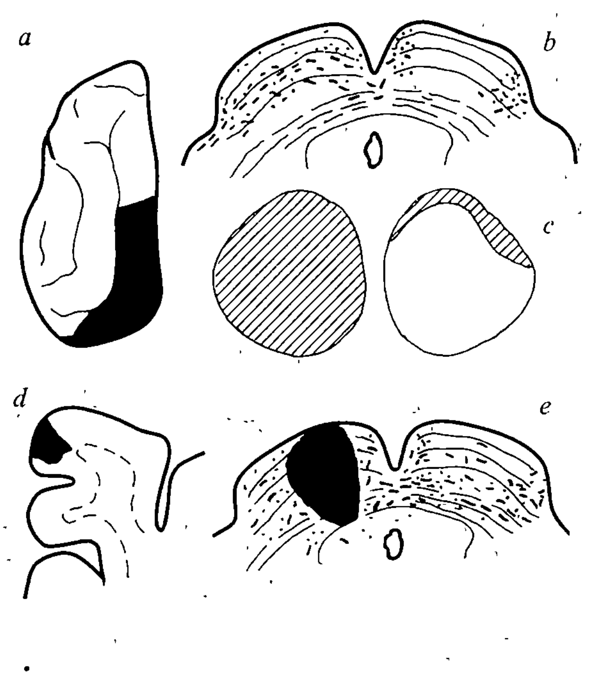
After a large lesion of the visual cortex with damage to or undercutting of areas 17, 18 and 19, there is fibre and terminal degeneration in the superficial four layers of most of the extent of the superior colliculus on the same side of the brain (Fig. 1*a* and *b*). The features of this degeneration are similar to previous descriptions based on the Nauta–Gygax method. There are horizontally disposed fibre fragments and fine granular degeneration in the stratum zonale; in the stratum griseum superficiale the degeneration is very dense in its deep half and much less in its superficial part;

in the stratum opticum the fibre fragments are coarser with less terminal degeneration, whereas in the stratum griseum intermediale the intensity of the degeneration is less and diminishes progressively towards its deep margin. On the contralateral side there is degeneration in the same superficial layers over a restricted area close to the medial and lateral margins. It is present over approximately the rostral half of the lateral margin, affects rather less of the medial margin and some continues round the rostral end of the colliculus (Fig. 1*b* and *c*); this part of the superior colliculus contains the representation of the midline of the retina[2-4]. The intensity of the degeneration is less than that of the operated side and is most evident in the stratum opticum and stratum griseum superficiale. Degeneration on the contralateral side is also found after smaller lesions of the visual cortex, and when the lateral half only of the ipsilateral colliculus contains degeneration, as after damage of the anterior half of the visual cortex, the degeneration on the contralateral side is found only at the lateral margin. Even with relatively small lesions at the boundary of areas 17 and 18 a few fibre fragments are found in the contralateral colliculus and always at a site corresponding to that on the operated side.

In three Siamese cats with lesions in the visual cortex degeneration is also present close to the margins of the anterior half of the contralateral superior colliculus as well as on the ipsilateral side. In one of these cats, the lesion was virtually restricted to the cortex related to the aberrant projection of the 20° of the ipsilateral visual field[7] (Fig. 1*d*).

The fibres which terminate in the contralateral colliculus probably reach that side after passing through the ipsilateral colliculus. The evidence in support of this is that no fibre degeneration has been seen in the brachium of the superior colliculus on the contralateral side, but a few degenerating fibres can be seen passing through the deeper layers of the superior colliculus on the operated side into the commissure of the superior colliculus. In addition, in several cats with lesions restricted to the superior colliculus of one side there is severe degeneration throughout the deeper layers of the contralateral superior colliculus with less in the stratum opticum, in most of the extent of the stratum griseum superficiale there is only an occasional fragment and there is none in the stratum zonale. In those parts containing the representation of the midline of the retina, and in which the fibres from the contralateral visual cortex terminate, the distribution and intensity of the degeneration is the same as after lesions of the cortex (Fig. 1*e*).

Fig. 1 The distribution of the fibre (dashes) and terminal (dots) degeneration in the superior colliculus of the two sides of the midbrain, in a coronal section (*d*) and (as hatching) on an orthographic reconstruction (*c*), after a large lesion (solid black) of the visual cortex (*a*). The solid black in (*d*) shows the site of the lesion in the representation of the aberrant 20° of the visual field of a Siamese cat, which also resulted in fibre and terminal degeneration in the contralateral superior colliculus. The solid black in (*e*) shows the site of a lesion restricted to the superior colliculus of one side and the resulting degeneration in a coronal section of the midbrain.



The finding of the projection from the visual cortex to the superior colliculus of both sides and the fact that the fibres to the contralateral nucleus end in the site of the representation of the midline of the retina may help to explain certain electrophysiological observations. It has been found in the cat that about 40° of the ipsilateral hemifield are represented in the rostral part of the colliculus and that this representation is binocular[8-10]. The pathway serving the representation of the ipsilateral hemifield has not been determined but it has been suggested that it is an indirect one and not direct from the retina[8,10]. The cells in the superficial layers of the representation of the ipsilateral hemifield had small receptive fields which were close to the midline and those in the deeper layers were large and extended further from the midline[10]. On the basis of these findings we suggest that the pathway for the ipsilateral hemifield representation to the superficial layers is through the projection from the contralateral visual cortex and that to the deeper layers is from the superior colliculus of the opposite side. The finding of a similar crossed cortical fibre pathway in the Siamese cat is interesting because the representation of the ipsilateral hemifield seems to be related to the opposite eye only[10], and because some fibres at least of the pathway to the opposite colliculus seem to arise either in the part of the cortex related to the aberrant projection of the 20° of the visual field or in the immediately adjoining part of the visual cortex. Finally, as the superior colliculus is now known to send fibres to the lateral geniculate nucleus and other thalamic nuclei[11,12], the contralateral projection to the opposite superior colliculus from the visual cortex could serve as an indirect pathway from the visual cortex of one hemisphere to that of the other hemisphere even after section of the corpus callosum and other forebrain commissures.

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Received December 18, 1975, accepted February 18, 1976.

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Size accentuation in the dominant eye

CONTRARY to popular opinion, people do not use their two eyes equally. It has been found that in tasks where only one eye can be used (such as sighting down a telescope) ~65% of all observers consistently select the right eye, 32% the left and only 3% are ambiocular[1-4]. This preference[5] occurs even when both eyes are open, as when one points to a distant object with the finger-tip. If one alternately closes each eye, it will be apparent that the distant target is aligned with the dominant eye but not with the other. Here we report investigations into whether there is a difference in the apparent size of stimuli presented to the dominant and non-dominant eye. We found that ~2/3 of our subjects see the object as being bigger with their dominant eye.

A sample of 45 observers was pretested to verify equal acuity in the two eyes. Each was screened to determine sighting dominance using two tests. The first dominance test was the Point Test, which involves having the observer point to the experimenter's nose with both eyes open. The experimenter notes which eye is aligned with the finger. Four repetitions were administered with the observer alternating hands to control for manual dominance. The second procedure for assessing eye dominance was the ABC Test, in which the observer holds the wide end of a truncated cone up to his face and views a target through the smaller aperture while keeping both eyes open. The cone must be squeezed in order to be held open, and the eye aligned with the aperture is scored as the dominant eye. There were four repetitions of this test. These procedures have been shown to be reliable indicators of the sighting dominant eye[1,3,5,6]. The sample was selected to contain 25 right-eyed and 20 left-eyed subjects.

Observers next viewed two white circular targets with a diameter of 10.5 cm at a distance of 40 cm through a haploscope which separated the inputs to the two eyes. They were asked to judge which of the two disks appeared 'larger' to them. This was a simple phenomenal judgment which all observers made without difficulty. Of the right-eyed subjects, 17 thought the object shown to their right eye to be larger, while of the left eyed, 13 thought objects presented to that eye the larger. In both cases, therefore, ~2/3 of the subjects thought that objects seen with their dominant eye were larger. These results are statistically reliable with a $\chi^2 = 4.90, P < 0.05$.

This difference is clearly psychological, rather than reflecting different magnification in the two eyes, since observers had been prescreened for equal refraction in both eyes. The reasons for these size distortions are not immediately clear. It seems likely that it may arise from image enhancement in the dominant eye or, perhaps, to differential selective attention to that image which gives rise to accentuation.

This research was supported in part by a grant from the Medical Research Council of Canada and from the National Research Council of Canada. The authors would like to acknowledge the assistance of Carole Behman, Michael Hewison, and Fred W. Whitford in the collection of the data.

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Possible role of surface Ig in non-random recirculation of small lymphocytes

THE selective extravasation of some lymph-borne immunoblasts in the gut-associated lymphoid tissue (GALT) is well established[1-5]. It seems that immunoblasts generated in the GALT return to it, whereas those formed in peripheral somatic lymph nodes (PSLNs) go to the spleen or other lymph nodes[6]. On the other hand, for want of contrary evidence, it is assumed generally that the small lymphocytes comprising the recirculating pool recirculate more or less randomly through the various nodes in the body. T and B lymphocytes may have different rates and microanatomical routes of recirculation through a given lymphoid organ[7-9] but until now it has not been possible to show that recirculating lymphocytes discriminate between different lymphoid organs. By comparing the recirculation of small lymphocytes from the intestinal lymph with that of lymphocytes from the efferent lymph of PSLNs in unanaesthetised sheep, we found that each population tends to recirculate preferentially through the type of lymphoid tissue from which the collections were made.

Crossbred adult wethers or ewes were anaesthetised and polyvinyl cannulae were inserted into the intestinal lymph duct and into an efferent duct of one or two of the PSLNs (popliteal, prefemoral or prescapular). After operation the conscious sheep were placed in metabolism cages and the lymph collected quantitatively[10-12]. Serial collections were made over periods of 8–16 h and in each experiment either a collection of intestinal lymph or a collection of efferent lymph was incubated *in vitro* with ^{51}Cr. The washed, labelled cells were returned to the sheep through an intravenous cannula after which regular samples of each type of lymph were taken for 24–48 h. The lymphocytes were washed, counted and radioassayed and their specific radioactivities determined. Eight such experiments were performed; four in which labelled intestinal cells were reinfused and four in which efferent cells from PSLNs were used (Fig. 1). Cells from intestinal lymph tend to reappear in that lymph, whereas cells from peripheral

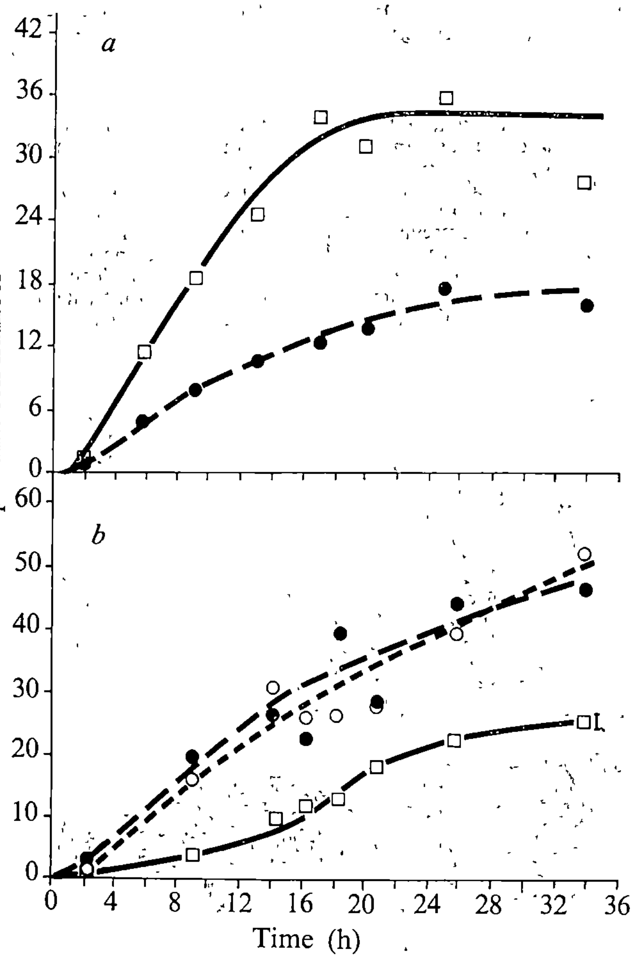

Fig. 1 The specific radioactivities (counts per unit cell number) of ^{51}Cr-labelled small lymphocytes in various lymphatic ducts after the injection, at time 0, of 3×10^9 cells from intestinal lymph (*a*) or 2×10^9 cells from prescapular efferent lymph (*b*). The cells had been labelled *in vitro* with Na$_2^{51}$CrO$_4$ at 15 μCi ml^{-1} for 1 h at 38 °C. □, Intestinal lymph; ●, prefemoral efferent lymph; ○, prescapular efferent lymph. Counting rates in individual samples were usually well above 10^3 c.p.m. except for the first few hours after injection of the labelled cells.

efferent lymphatics tend to recirculate through PSLNs; this happened quite unequivocally in every experiment.

In all experiments 2% of the injected labelled cells were immunoblasts; by labelling the immunoblasts with ^{125}I-iododeoxyuridine (^{125}IUdR) it was possible to compute the amount of ^{51}Cr associated with this type of cell and rule out the possibility that their presence could bias the results. The lower graph (*b*) confirms our previous report[13] that efferent cells from any one PSLN will recirculate equally well through any other PSLN. It should also be noted that there was no fall in the specific radioactivities of the lymph cells within the experimental period. This suggests a longer recirculation time for lymphocytes in sheep than in rodents[14], but is in agreement with other results from sheep[13,15].

It is obvious that the distinction between peripheral and intestinal recirculation is by no means absolute. This may indicate that there is only a tendency for a given cell to return to its place of origin, but the results could just as well be explained by the presence of two populations; one made up of cells with a strong preference for a given pathway, and the other made up of randomly recirculating cells. The return of immunoblasts to the GALT is possibly mediated by their immunoglobulin A (IgA) or a closely associated molecule[6,14]. It seems plausible, therefore, that those small lymphocytes which recirculate preferentially through the GALT do so because of their α-associated determinants, as up to 40% of all Ig-bearing lymphocytes are believed to have α chains on their surfaces[17]. Thus, we suggest that the route of recirculation of B cells may be influenced by the class of their surface Ig, while the non-Ig bearing T cells recirculate randomly. Sordat *et al.*[18] found significant amounts of Ig in the walls of post-capillary venules in the tonsil and although functional evidence was lacking they suggested that the presence of Ig was in some way related to lymphocyte recirculation. If these ideas are true it is possible that the number of B cells available to a given tissue may be limited to those with the corresponding Ig subclass; this could be important in determining the nature of immune responses and possibly also the metastatic pattern of some malignant lymphomata.

We acknowledge the technical assistance of David Glover and support from the MRC and Cancer Research Campaign.

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Received December 12, 1975; accepted February 19, 1976.

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Serum-dependent adhesion and cytotoxicity of cells to *Litomosoides carinii* microfilariae

SERA from infected or immunised hosts have been reported to promote adhesion of leukocytes from those hosts to a variety of parasitic helminths[1–5]. Higashi and Chowdhury[6] found selective adhesion of eosinophils to the infective larvae of *Wuchereria bancrofti* in the presence of serum from filariasis patients showing microfilaraemia, lymphoedema or elephantiasis. In albino rats infected with the filarial parasite *Litomosoides carinii* a termination of microfilaraemia (onset of latent infection) is associated with the adhesion of cells to microfilariae in the pleural cavity where the adult worms reside. Cells which seem morphologically to include macrophages, lymphocytes and polymorphs can be seen attached to living as well as to disintegrating microfilariae[7]. Virtually no attachment is seen during the patent stage of the infection. Furthermore, immunosuppression of rats with latent infection by means of cortisone, cyclophosphamide anti-lymphocyte serum or whole-body irradiation can lead to the appearance of microfilaraemia in the animals[7–9]. Because the observations suggest the occurrence of an immune reaction, perhaps cell mediated, against microfilariae we investigated the mechanism of cellular adhesion. The results reported here show that adhesion *in vitro* is mediated by a serum factor and is accompanied by a cytotoxic effect on microfilariae.

Microfilariae were isolated from heparinised blood of *L. carinii*-infected rats by sedimentation of blood cells with dextran (molecular weight 200,000–275,000). They were washed and resuspended in tissue culture medium (HEPES-buffered RPMI1640 (Gibco)), containing penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). They were then counted in a haemocytometer. Spleen cells were prepared by teasing the tissue in medium and were washed twice before use. The peritoneal cavities of normal rats were washed out with 10 ml of the medium containing heparin (10 U ml^{-1}) and the cells were cultured in ring-and-slide chambers[10]. The non-adherent cells were washed off after incubation for 2 h at 37 °C, leaving cultures of peritoneal macrophages. The sera used were obtained from normal rats, from rats with patent infection and from rats with latent infection (samples from eight rats in each group) and from rats and rabbits hyperimmunised with homogenates of adult parasites in Freund's complete adjuvant (samples from two rats and two rabbits).

For adhesion experiments the reaction mixtures contained microfilariae and spleen cells at a ratio of 1 : 500–1 :2000 in a volume of 0.5 ml of medium with 20% rat serum. They were incubated at 37 °C for 16 h with occasional shaking A drop of the mixture was examined microscopically and the percentage of microfilariae having cells attached calculated. Adhesion occurred only when the mixture contained serum from rats with latent infection. Some typical results are presented in Table 1, which shows that spleen cells from rats with latent infection can become attached only in the presence of latent rat serum and that cells from mice and guinea pigs, but not man, can become attached, although to a lesser degree. Other experiments showed that adhesion was appreciable at 6 h and had reached a maximum by 16 h incubation. In general, adhesion was not seen in mixtures containing less than 10% latent rat serum. In

Table 1 Adhesion of spleen cells to microfilariae

Source of cells	Source of serum	% Microfilariae with spleen cells attached
Normal rat	Latent rat	80
	Normal rat	2
	Infected rat	4
	Immunised rat	0
	Immunised rabbit	0
Latent rat	Latent rat	75
	Normal rat	4
Mouse	Latent rat	54
Guinea pig	Latent rat	17
Human*	Latent rat	0

*Peripheral leukocytes were used in the experiments.

Table 2 Role of complement in adhesion

Experiment	Treatment of latent rat serum	% Microfilariae with spleen cells attached
1	None	92
	Heated (56 °C 30 min)*	0
	Heated+NRS†	39
2	None	78
	HSA–anti-HSA*	10
	HSA–anti-HSA+NRS†	20

*No haemolytic complement activity detectable.

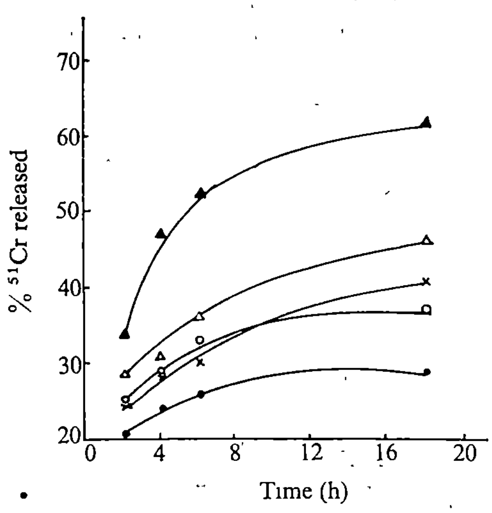
†Amount of normal rat serum (NRS) used completely lysed 10^9 antibody-sensitised sheep red blood cells. Mixture contained 20% latent serum and 20% NRS. HSA, Human serum albumin.

other experiments spleen cells which had been passed through a glass wool column[11] became attached to only 15% of microfilariae in the presence of latent rat serum, whereas virtually all microfilariae become attached to peritoneal macrophages after incubation in the presence of latent rat serum.

On the assumption that immunoglobulin antibodies were required for adhesion, the role of complement was investigated. Table 2 shows that latent rat serum decomplemented by heat (56 °C, 30 min) or by an antigen–antibody precipitate failed to induce adhesion and that its capacity to induce adhesion was only partly restored by normal rat serum. The poor restoration in the second experiment may have been due to persistence of small amounts of immune complexes, which are potent inhibitors of reactions between cells and antibody-treated targets[12]. These observations may indicate the operation of two mechanisms for inducing adhesion, one involving antibody only and one involving antibody plus complement.

Further experiments were carried out to determine whether lymphoid cells damaged microfilariae in the presence of latent rat serum. Microfilariae liberated from washed adult worms during incubation (37 °C, 1 h) in medium were labelled with ^{51}Cr essentially as described by Butterworth *et al.*[13] for schistosomulae, except that tissue culture medium containing 20% normal rat serum was used. They were then washed five times in medium and resuspended in medium containing normal rat serum. The suspension contained 3,000 microfilariae per ml, giving about 300 c.p.m. per microfilariae. They were incubated with normal spleen cells and with additional serum (to a concentration of 20%) at 37 °C. The release of ^{51}Cr was measured at intervals. Figure 1 shows that there was a marked increase in ^{51}Cr release over control levels in the presence of latent rat serum and normal spleen cells.

Fig. 1 Release of ^{51}Cr from labelled microfilariae on incubation with normal spleen cells and latent rat serum. ▲, Normal spleen cells (NC) + latent rat serum (LRS); △, NC + normal rat serum (NRS); ×, NRS; □, LRS; ●, medium.



Our earlier observations[7–9] suggested that cell-mediated immunity may be important in the destruction of microfilariae. The experiments described here implicate both humoral and cellular factors in a collaborative reaction, which may be similar to that directed against schistosomules.

D.S. received an exchange of research workers grant from the World Health Organization. Work in the Kolling Institute was supported in part by grants from the National Health and Medical Research Council and the Postgraduate Medical Foundation of the University of Sydney. We thank Cynthia Shneider and Jean Penrose for assistance.

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HeLa cells secrete α subunit of glycoprotein tropic hormones

THE HeLa line, derived from a carcinoma of the cervix, was the first continuous human tissue culture line to be established *in vitro*[1], and has been one of the primary sources for the study of human cell biology. Although such studies have documented the biosynthesis of certain intracellular and membrane proteins[2–4], there has been little specific characterisation of proteins secreted by these lines The human glycoprotein tropic hormones, thyroid-stimulating hormone (HTSH), follicle-stimulating hormone (HFSH), luteinising hormone (HLH), and chorionic gonadotropin (HCG), are each composed of two subunits—an immunologically indistinguishable and probably common α subunit ('α') and distinct β subunits which confer biological specificity on the complete molecule[5–8]. Complete hormones and free, isolated subunits are secreted by their normal cells of origin in pituitary or placenta under a variety of physiological and neoplastic circumstances[9–11]. Furthermore, complete hormones and isolated subunits can even be secreted ectopically—that is, by tissue which does not in normal conditions produce these proteins. Since ectopic α secretion has been documented for a variety of neoplasms, both *in vivo*[12] and *in vitro*[13], we examined the possibility that HeLa lines might also ectopically secrete the α subunit. We have found that three different HeLa strains secrete a substance immunologically indistinguishable from the α subunit, with secretion rates differing by as much as 50-fold

In identical laboratory conditions we have grown

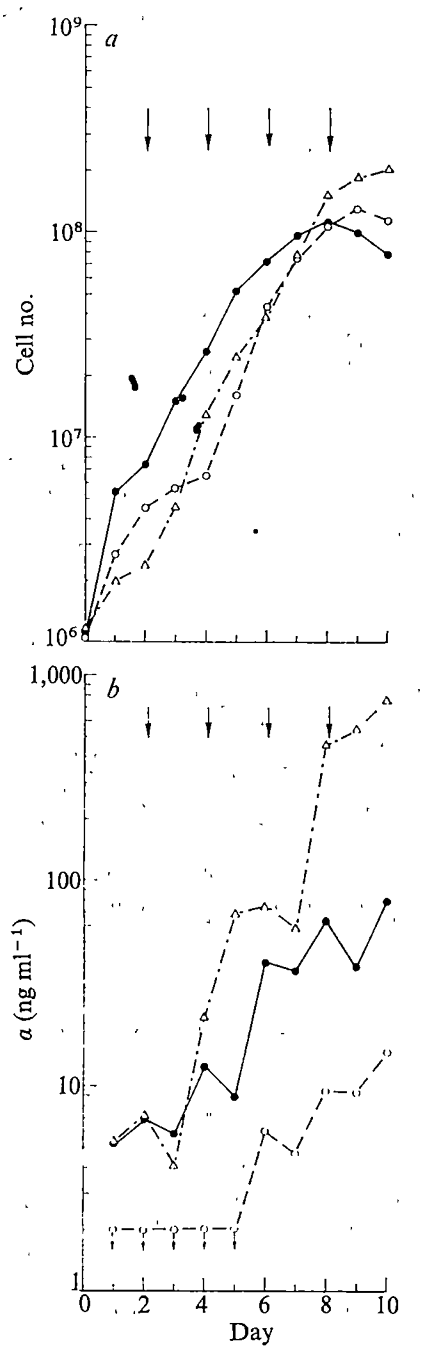

Fig. 1 Growth curves and α concentrations for the three strains: ●, HeLa CCL 2; ○, HeLa CCL 2.1; △, HeLa CCL 2.2. All cultures were established at day 0, and for each strain two cultures were used on each day of measurement. *a*, Total cell number per 75-cm^2 flask; *b*, media concentrations of α measured in the same double-antibody radioimmunoassay using HCG-α as reference standard. Arrows indicate days when the medium in each remaining flask was exchanged with fresh medium; α concentrations on those days were measured immediately before medium change. HeLa CCL 2 has also been called HeLa or HeLa(Gey), HeLa CCL 2.1 has been called HeLa 229, and HeLa CCL 2.2 has been called HeLa S$_3$

sequential separate cultures of three HeLa strains—HeLa CCL 2, 2.1 and 2.2 (American Tissue Culture Collection)[14]. Each culture was grown at 37 °C in 75 cm^2 flasks in 30 ml α minimal essential medium with 10% foetal calf serum. On each day of measurement, two cultures of each strain were used. On each day, we counted cell number after trypsinisation and measured α concentration in the culture media by a specific double-antibody radioimmunoassay using HCG-α as a reference standard[12]. As the cell numbers increased, all three lines progressively accumulated α immunoreactivity in the media, although in markedly different concentrations (Fig. 1). At no time was HCG-β detected by radioimmunoassay (<0.2 ng ml^{-1}) in the media[15]. Complete glycoprotein hormones containing α subunit have small (1–3%) cross reactions in the α assay[12], and a very large amount of any of these hormones could have accounted for the α immunoreactivity. This possibility was excluded by examining the media in specific radioimmunoassays for HTSH, HLH, HFSH and HCG[12,16,17]; all were undetectable (HTSH <1.0 μU ml^{-1}, HCG <0.2 ng ml^{-1}, HLH <1 mIU ml^{-1}, HFSH <1.5 mIU ml^{-1}). Control medium not exposed to HeLa cells had undetectable amounts of α (<2 ng ml^{-1}). Media exposed to HeLa cells had no effect on ^{125}I-HCG-α tracer integrity, since such tracer was still fully immunoprecipitable by excess antibody to HCG-α. The peak secretory rates of α by HeLa 2.1 was 0.1, by HeLa 2 was 1.0 and by HeLa 2.2 was 5.0 pmol per 10^6 cells per day.

The ectopic secretion of α by cell lines derived from a carcinoma of the cervix is of interest. Whether the original tumour secreted α *in vivo* is not known. Certain tumours and cultures are known, however, to secrete progressively more α subunit *in vitro* with time[9]. Indeed, the K_1 clonal strain of the ChaGo bronchogenic carcinoma cell line secretes 290 pmol α per 10^6 cells per day, substantially more than the wild type[18].

Serial dilutions of alpha in the HeLa tissue culture medium exactly paralleled standard curve dilutions of HCG-α in the radioimmunoassay, indicating virtual immunological identity. The gel chromatographic behaviour of HeLa-α was also studied (Fig. 2). Medium from a confluent culture of HeLa 2.2 was cochromatographed on Sephadex G-100 with ^{131}I-HCG-α as an internal marker. This procedure discriminates between the glycoprotein hormones and their subunits, and on this column the α subunits of HTSH, HFSH, HLH and HCG all elute identically[19]. The HeLa-α eluted in the α subunit region, again clearly differentiating it from the complete glycoprotein hormones. Although the elution pattern of HeLa-α is similar to that of HCG-α, HeLa-α does have a slightly higher apparent molecular weight. In this respect, HeLa-α is similar to the α produced by a gastric carcinoid *in vivo* and a bronchogenic carcinoma *in vitro*[19]. The very similarity in α produced by these diverse neoplasms raises the possibility, among several other explanations, that tumour α may be a precursor of normal α (ref. 19).

All three strains of HeLa studied secreted α, in fact two different cultures of HeLa 2.2 obtained from different sources (American Type Culture Collection and Flow Laboratories) had almost identical α-secretion rates. It has been suggested that many human tissue culture lines have been overgrown and replaced by HeLa cells[20–22]. Although α secretion is not exclusive for HeLa—certain cell lines such as ChaGo, which are not HeLa by karyologic criteria[18], secrete α—the presence of α secretion by a human cell line should prompt careful examination for known karyologic and enzymatic HeLa markers. For example, we have found significant quantities of α (14 ng ml^{-1}) in the medium of HBT$_3$, a line originally derived from a breast cancer which has been found to have karyologic HeLa markers[22]. On the other hand, it is not known whether the absence of α secretion rules out HeLa. Three different HeLa strains, including two different sources of HeLa 2.2, secreted α, but in the case of HeLa 2.1 we could only detect α at high cell density after a number of days. We do not know, however, whether all HeLa strains secrete α even at high density after many days.

It has been reported that another HeLa strain, HeLa$_{65}$—a variant of HeLa CCL 2.2—secretes very small amounts (0.0024 ng per 10^6 cells per day) of material which has activity in an HCG-β immunoassay[23]. This basal secretion

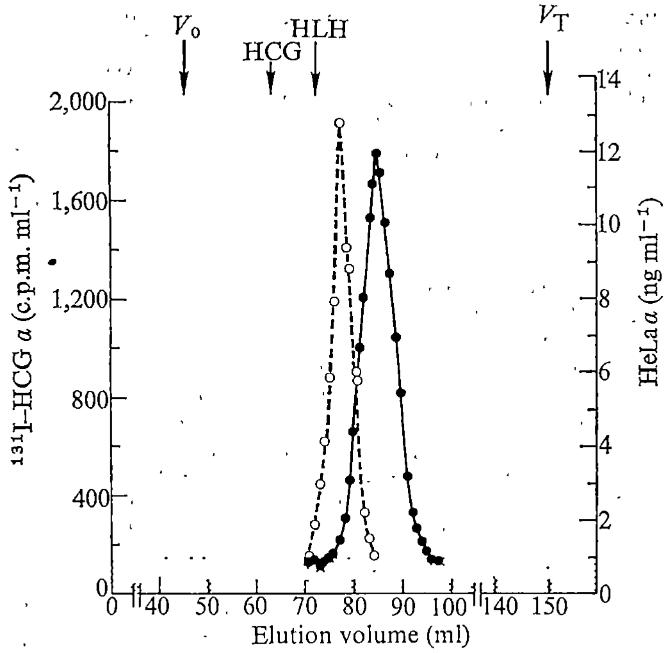

Fig. 2 Gel chromatography of ^{131}I-labelled α subunit of chorionic gonadotropin (^{131}I-HCG-α) added to medium from a confluent culture of HeLa CCL 2.2. The Sephadex G-100 column (1.5×90 cm) was equilibrated at 4 °C and eluted with 0 15 M NaCl, 0.01 M Na phosphate, *p*H 7 4, at a flow rate of 5 ml h^{-1}; 1-ml fractions were counted for ^{131}I radioactivity (●) and measured for α immunoactivity (○) by a ^{125}I radioimmunoassay. The shaded area is below the detection limits of the radioimmunoassay The void volume (V_0) and the total volume (V_T) were determined in the same chromatogram. In separate experiments HCG and HLH migrated with elution volumes as indicated by the arrows. The column was calibrated with five standard proteins (apoferritin, gammaglobulin, ovalbumin, chymotrypsinogen A, and ribonuclease). HeLa-α had an apparent molecular weight of ~33,000, whereas HCG-α had an apparent molecular weight of ~24,000 The molecular weight calculated from the chemical composition of purified HCG-α (15,000) was used to calculate the secretion rates in the text. Differences between the apparent molecular weight of α and that calculated from chemical composition have been discussed elsewhere[19].

rate is four orders of magnitude less than the α secretion (75 ng per 10^6 cells per day) by HeLa CCL 2.2 reported here. Although this reported HCG-β immunoactivity (HCG and/or HCG-β) may to some extent be accounted for by cross reaction with large access of free α, it has been observed that certain ChaGo clones which secrete large amounts of free α also secrete small amounts of biologically active HCG[13].

We do not yet know what relation α secretion by these HeLa strains has to their different karyotypes or even to intracellular production of other HeLa proteins such as placental alkaline phosphatase. The fact that three HeLa strains derived from a carcinoma of the cervix have widely different secretion rates of α suggests that they may be useful in identifying the factors controlling the production of this protein, which is normally repressed in cervical cells.

A number of diverse neoplasms have been shown to secrete the α subunit ectopically *in vivo* and/or *in vitro*; these include cancers of lung[13], stomach[12], pancreas[12], and now uterine cervix Studies of α secretion should be extended to other human cell lines and, when assays become available, to cell lines from other species. Perhaps the α subunit will turn out to be a relatively easily derepressed and 'primitive' protein.

We thank Dr Arlene Levine for supplying HBT₃ medium, Ms Ida Stotler for assistance, Dr Richard Dimond for measuring HTSH, and Drs Nimai Ghosh and Rody Cox for sending us their preprint.

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Interference between anti-HLA antibodies and chlorpromazine

IT has been suggested that the effects of chlorpromazine in man and animals are genetically influenced[1,2]. In mice, genetic control of sensitivity to this drug seems to reside in two loci, the more important of which lies on chromosome 9 at about the region which controls the histocompatibility system H2 (ref. 3). We have investigated whether the presence or absence of some alleles of the HLA system—analogous to the H2 system of mouse—correlate with individual sensitivity to the drug. We evaluated the clinical responses to chlorpromazine of 33 chronic schizophrenic subjects who had been HLA typed during previous investigations[4,5]. There was a highly significant positive response to chlorpromazine in patients with the antigen HLA-A1 of the HLA-A locus (unpublished results of E.S., L. Bellodi and E. Sacchetti). A possible explanation of the correlation is that the presence of HLA-A1 on the cell membrane makes chlorpromazine bind more readily to the cell. As chlorpromazine binds to lymphocyte membranes[6], we investigated whether or not the binding of the drug could interfere with the specific binding of anti-HLA antibodies, particularly anti-HLA-A1 antisera. Furthermore, as chlorpromazine binds to the β-adrenergic cell receptors, which are also present on peripheral blood lymphocytes[7], we also used the β-adrenergic mediators dopamine (DA) and noradrenaline (NA).

Five healthy volunteers were selected on the basis of their HLA types from our HLA reference panel. Three were HLA-A1 positive and two were negative for the

Table 1 Effect of chlorpromazine, DA and NA on absorption of two anti-HLA-A1 sera and serum MI P1 (anti-HLA-B_{12},B_{13}) by human peripheral blood lymphocytes

	MI P1					MI P42					Morrison				
	A 1,9,7,12	B 1,5,8	C 1,12,8	D 2,5,12	E 9,12	A 1,9,7,12	B 1,5,8	C 1,12,8	D 2,5,12	E 9,12	A 1,9,7,12	B 1,5,8	C 1,12,8	D 2,5,12	E 9,12
Original cytotoxic titre	1/8	—	1/8	1/16	1/8	1/16	1/8	1/16	—	—	1/16	1/16	1/16	—	1/4
Cytotoxic titre after absorption with fresh lymphocytes	—	—	—	—	—	1/2	—	—	—	—	—	—	—	—	—
Cytotoxic titre after absorption with lymphocytes preincubated with															
Chlorpromazine (0.4×10^{-4} M)	1/8	—	1/8	1/16	1/8	1/16	1/8	1/16	—	—	1/8	1/8	1/16	—	—
DA (0.8×10^{-4} M)	1/8	—	1/8	1/16	1/8	1/4	1/8	1/8	—	—	1/8	1/8	1/8	—	—
NA (0.8×10^{-4} M)	1/8	—	1/8	1/16	1/8	1/4	1/4	1/8	—	—	1/4	1/8	1/8	—	—

Results are expressed as the serum dilution giving a cytotoxicity >80%.

same antigen. Each subject was typed many times in different typing sessions and tested with the antisera used in this work. The types were: G.C.: HLA-A1, A9, B7, B12; L.B.: HLA-A1, B8, B12; L.M.: HLA-A1, B5, B8; B.P.: HLA-A9, B12; R.S.: HLA-A2, B5, B12. Thirteen anti-HLA antisera were selected from sera supplied from the NIH bank (Bethesda) and from cytotoxic sera from multiparous women screened in our laboratory for their specificity and compared with highly selected antisera from the Institute of Medical Genetics of the University of Torino. The sera, with their corresponding specificities were: Morrison: anti-HLA-A1, A9, B8; MI P42: anti-HLA-A1, AW25; MI P80: anti-HLA-B8, AW32+; MI P106: anti-HLA-A2; MI P138: anti-HLA-A2; MI Pt1 18/3: anti-HLA-A9; MI P145: anti-HLA-A9; D666222IX: anti-HLA-B5; MI P71: anti-HLA-B5+; MI P55: anti-HLA-B7; MI P128: anti-HLA-B7; MI P1: anti-HLA-B12, B13; MI P109: anti-HLA-B12+.

Each antiserum was tested for its cytotoxic titre against lymphocytes bearing the corresponding HLA specificity. Lymphocytes (6×10^{-6})—obtained from heparinised peripheral blood by flotation on Lymphoprep[8]—were incubated in Hanks' solution (buffered at *p*H 7.2 by adding isotonic bicarbonate solution) containing chlorpromazine or DA or NA at a concentration of 1.5×10^{-4} M and rocked at 2 °C for 1 h. Afterwards, tubes were centrifuged at 0 °C for 15 min at 600*g*, the supernatant layers were discarded and the cells washed once with cold Hanks' solution. Then, the washed lymphocytes (6×10^{6}) were resuspended in 0.3 ml of HLA cytotoxic antisera and incubated again at 2 °C for 1 h. Controls were carried out by incubating the same amount of cytotoxic antisera with 6×10^{-6} lymphocytes which had not been preincubated with the drugs. Afterwards tubes were centrifuged at 600*g* for 15 min at 0 °C and the supernatant layers, after a further centrifugation at 2,000*g*, were tested for their cytotoxic titre on fresh lymphocytes from the donors used for the absorption study.

We found no difference between the cytotoxic titres of antisera absorbed by control lymphocytes and by lymphocytes preincubated with drugs, except for the anti-HLA-A1 sera MI P42 and Morrison when tested with HLA-A1 positive cells (Table 1). Morrison serum showed no variation when it identified the antigen HLA-A9 on HLA-A1 negative cells (donor B.P.).

We also made a dose-inhibition study to determine the lowest concentrations of the drugs we were investigating, which were able to cause the greatest inhibitions of anti-HLA-A1 antisera.

We found that chlorpromazine was active at 0.4×10^{-4}, whereas DA and NA had their greatest effects at 0.8×10^{-4} M. At a higher concentration of chlorpromazine (0.6×10^{-3} M) we observed slight inhibition of two other antisera not detecting HLA-A1—sera MI P109 and MI P71. At the same concentration, DA and NA were ineffective.

Our data suggest specific binding of chlorpromazine to the antigen HLA-A1 of the first HLA locus which interferes with the specific binding of the anti-HLA-A1 immune sera. DA and NA also seem to bind to the HLA-A1 antigen, but perhaps with a lower affinity than chlorpromazine, as greater concentrations were needed to exert less inhibition than that due to chlorpromazine.

We think two interpretations of our results are possible. First, the HLA antigens could be structurally linked to the β-adrenergic lymphocyte receptors in the cell membrane grid. HLA-A1 might modify the quaternary structure of these receptors, making it easier for chlorpromazine to bind it and making possible a reciprocal steric inhibition of anti-HLA-A1 antiserum and chlorpromazine or DA and NA. The other HLA antigens, less intimately connected with the β-adrenergic receptors, show no such reciprocal interference. There is another possibility as many cell membrane structures are originally derived from a common ancestor. For example, all the structures governed by the HLA region are phylogenetically related[9,10]. Moreover, the structural analogies between the immunoglobulins, the HLA antigens and β_2-microglobulin have also suggested a common evolutionary origin for these molecules[12].

Thus we think it possible also that β-adrenergic receptors and HLA antigens descend from a single precursor and that HLA-A1 is the antigen which is less divergent from this common original structure and from the cell β-adrenergic receptor than the other HLA specificities. For this reason it would be interesting to investigate the relationships between such receptors and those HLA antigens which cross react with HLA-A1.

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Received November 24, 1975; accepted January 30, 1976.

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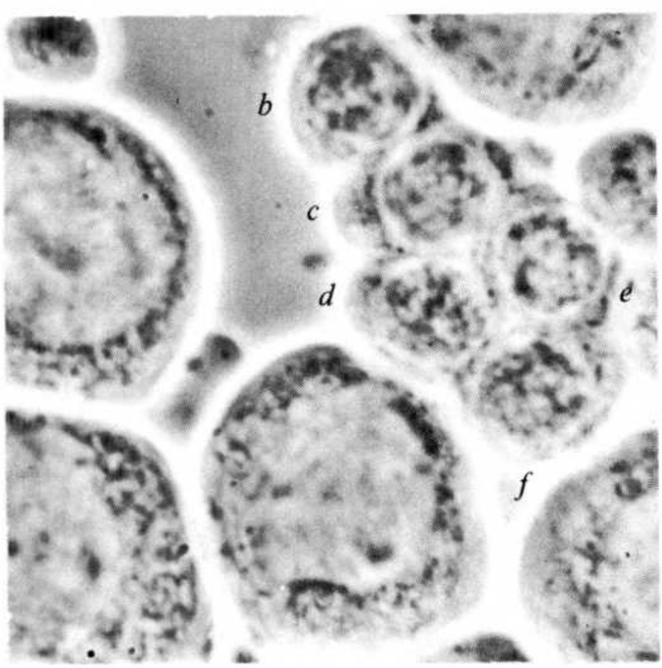
Chromosome rotation and formation of synapsis

ALTHOUGH the rotation and oscillation of the nuclei of cultured spermatocytes have been known for some years[1,2], no correlations of this phenomenon with the stages of mitosis or meiosis, or with the biochemical or cytogenetic events during germ-cell development have been made. Using a new technique for the rapid separation of segments of rat seminiferous tubules containing accurately known cellular associations and stages of meiotic prophase[3] we have been able to correlate the rotation of living spermatocyte nuclei with synapsis during rat spermatogenesis.

Immediately after removing the testes from the animal, the seminiferous tubules were removed and transilluminated under a stereomicroscope. Cells representing different stages of meiotic prophase were selected for analyses with time lapse phase-contrast cinemicrography[4]. Figure 1 shows a phase-contrast photomicrograph of early zygotene spermatocytes developing synchronously at stage XII of rat spermatogenesis. The chromosomes of cell *c* are not sharp in the figure, indicating chromosome movement during the 4-s exposure time. That these structures really are chromosomes, was shown by Pleshkewych and Levine[5] using electron microscopic preparations of mouse primary spermatocytes, previously analysed by phase-contrast microscopy.

At the beginning of meiotic prophase at stages IX–X (ref. 6) no movements were observed in the chromosomes of the leptotene spermatocytes. In late leptotene spermatocytes, at stage XI, some nuclei were seen rotating, and an oscillatory movement of the chromosomes was common. At the next stage (XII) the early zygotene spermatocytes showed the most active rotations and oscillations of their chromosomes. The movements then gradually slowed down towards the end of zygotene stage (XIII–XIV) and ended during the early pachytene stage (before stage III). Some oscillatory movements of the chromosomes were seen up to stage VI, but mid- and late-pachytene spermatocytes (stages VII–XII) showed no chromosomal movements.

Fig. 1 Phase-contrast photomicrograph of early zygotene spermatocytes developing synchronously at stage XII of rat spermatogenesis. Seminiferous tubular fragments containing accurately identified cellular associations and stages of meiotic prophase were separated using a variation in the transillumination pattern of freshly isolated unstained seminiferous tubules as a marker for different stages of spermatogenesis[3]. The cells were squeezed to form a monolayer between object and cover glass slides, and observations were made under phase-contrast optics. The rotational pattern of the cells *b*–*f* are analysed in corresponding tracings in Fig. 2. Owing to the 4-s exposure time, the chromosomes of cell *c* have moved and are unsharp in the figure. Note the size difference of the late pachytene spermatocytes.



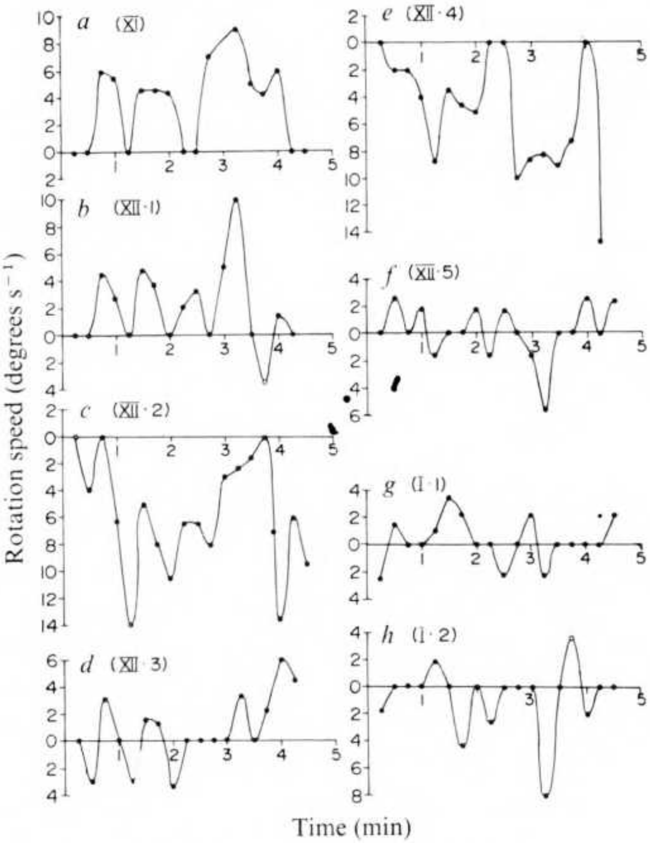


Fig. 2 Analysis of the rotating movements of the chromosomes of rat primary spermatocytes at different stages of development. Time-lapse cinemicrographs were taken at a speed of 2 frames s^{-1} with a Beaulieu 4008 ZM camera using super-8 film. The film was analysed using a viewer, which makes it possible to trace the interesting frames and to measure the time intervals. All observations were made within 4 h of killing the animal and 3.5 h of sealing the specimen chamber. The rotation speed is indicated as degrees s^{-1} at the ordinate, clockwise direction above the abscissa and counterclockwise direction below it. The observation time (min) is indicated on the abscissa. The movement is not even, and most cells change the rotation direction several times during the observation. The maximum speed (cell *e*) is 14.6 degrees s^{-1}. Tracings *b*–*f* are for cells belonging to the same synchronously developing group of cells depicted in Fig. 1.

In individual cells, the speed and direction of the rotational movement varied considerably. Figure 2 shows an analysis of cells from early zygotene (stages XI and XII) and early pachytene (stage I) primary spermatocytes. The chromosomes were followed on time-lapse film and traced every 15 s, and the rotation angle was measured. The clockwise rotation speed (degrees s^{-1}) is seen above the abscissa and the counterclockwise rotation speed below it. The cells were followed during 4.5 min (abscissa). The speed of the nuclear rotation was not even in any cell, and in most cases the direction of the movement changed during the observation time. In addition, the chromosomes seemed to move around several axes in numerous different planes. This is not shown in the tracings. Even in synchronously developing clones of early zygotene spermatocytes (Fig. 1) the rotation speed and direction vary considerably between individual cells (Fig. 2, *b*–*f*).

It is generally known that during zygonema (stages XII–XIV in rat spermatogenesis) the homologous sets of sister chromatid pairs begin to come together and associate with one another. The result is a precise point-for-point alignment, synapsis. The fully developed apparatus, the synaptinemal complex, is completed during pachynema. The rotational movements of the chromosomes seem to correlate to the formation of synapsis, so that in zygonema, where the synapsis is formed, the chromosomal movements are the most rapid. In early pachynema when the synapsis is completed there is still some movement of the chromosomes, but when the synaptinemal complex is fully developed the movements of the chromosomes cease completely. The movements of the chromosomes may be helpful in the physical mutual recognition of the specific parts of chromosomes, leading to the formation of synapsis between homologous chromosomes.

We thank Dr Hans Carstensen for encouragement, and the Magnus Bergvall Foundation and the Finnish Research Council for Medical Science for support.

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Received November 12, 1975; accepted February 6, 1976.

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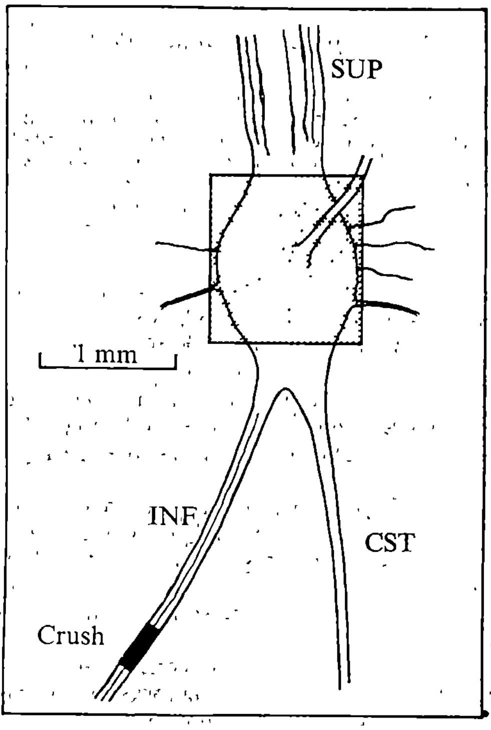
Effect of nerve growth factor on synaptic depression after axotomy

INTERRUPTION of the axon of a mammalian nerve cell leads to severe depression of synaptic transmission to the affected neurone within a few days, due primarily to loss of synaptic contacts[1–4]. In the superior cervical ganglion of the guinea pig, recovery of synaptic contacts on many neurones occurs if axon regeneration is allowed, but generally does not take place after axon ligation[4]. Furthermore, application of colchicine to postganglionic nerves causes synaptic loss in the absence of mechanical axon interruption[5] (see also ref. 6). These findings indicate that axonal extension to the periphery in some way provides the parent neurone with a 'trophic' signal which is instrumental in maintaining preganglionic synaptic contacts. In the sympathetic nervous system the protein nerve growth factor (NGF) has a number of effects on the metabolism and morphology of developing neurones whose survival is ultimately dependent on the presence of this agent[7]. One mechanism by which NGF affects neurones is an interaction with specific surface receptors on cell bodies[8]. More recent experiments have supported the suggestion[9] that NGF may also act as a messenger from the effector organ to innervating sympathetic neurones: NGF is selectively taken up by sympathetic endings[10], can be retrogradely transported to the neuronal somata[10,11], and has been shown to prevent some of the biochemical changes that follow axotomy in young animals[12,13]. Thus a natural question to ask is whether NGF might be involved in the regulation of synapses on mature ganglion cells. We report here that exogenous NGF can, to a large extent, prevent the synaptic depression seen in adult sympathetic ganglion cells after interruption of their axons.

Ten adult guinea pigs (200–400 g) were anaesthetised with pentobarbitone and both superior cervical ganglia exposed in aseptic conditions. On the right side, the inferior postganglionic nerve was crushed 2–3 mm from the ganglion; at the same time a silicone rubber (Silastic, Dow Corning) pellet measuring approximately 1.2 × 1 × 0.5 mm and containing 100–500 μg of purified 2.5S mouse salivary gland NGF[14] (from Dr R. A. Bradshaw) was implanted in contact with the ventral surface of the ganglion (Fig. 1). Pellets were prepared by mixing NGF with the silicone rubber before curing. On the left side, the inferior branch was crushed, but no pellet was implanted. In a further series of seven control animals, a silicone rubber pellet without NGF was implanted at the time of crushing the inferior postganglionic branch. After 4–7 d, the interval after which postaxotomy synaptic depression is maximal[4], ganglia were removed and studied by means of intracellular recording. Neurones on the dorsal surface of the ganglion were impaled with glass microelectrodes, and excitatory postsynaptic potentials (e.p.s.p.s) were measured in response to supramaximal stimulation of the preganglionic sympathetic trunk. Axons of individual neurones were determined to emerge in the crushed inferior branch, or the intact superior branch, by antidromic stimulation. The methods of impalement, stimulation, and e.p.s.p. measurement were as described previously[4].

In the animals in which a control pellet without NGF had been implanted, the amplitude distribution of e.p.s.p.s in neurones whose axons had been crushed (Fig. 2*b*) was shifted towards lower values, compared with the synaptic responses of normal neurones (Fig. 2*a*). This degree of synaptic depression is similar to that after crushing alone[4,5]. Thus, the presence of the control pellet had no effect on the course of postaxotomy synaptic depression. In each of 10 animals in which the implanted pellet contained NGF, however, the reduction in e.p.s.p. amplitude was curtailed; the synaptic responses elicited in most neurones whose axons had been crushed in treated ganglia fell within the normal range, whereas relatively few showed severe depression of synaptic transmission (Fig. 2*c*). In the course of studying NGF-treated ganglia, many nerve cells were impaled whose axons emerged in the intact superior branch (Fig. 1). Synaptic responses in these neurones were of normal amplitude (20–44 mV) suggesting that exogenous NGF has little effect on normal synaptic transmission. The input resistances, resting potentials, and action potential amplitudes were similar in treated and untreated control neurones whose

Fig. 1 Diagram of operative procedure. Ventral view of right superior cervical ganglion. CST, Cervical sympathetic trunk; INF, inferior postganglionic branch; SUP, superior postganglionic branch. Stippled area shows approximate size and location of silicone rubber pellet impregnated with NGF.



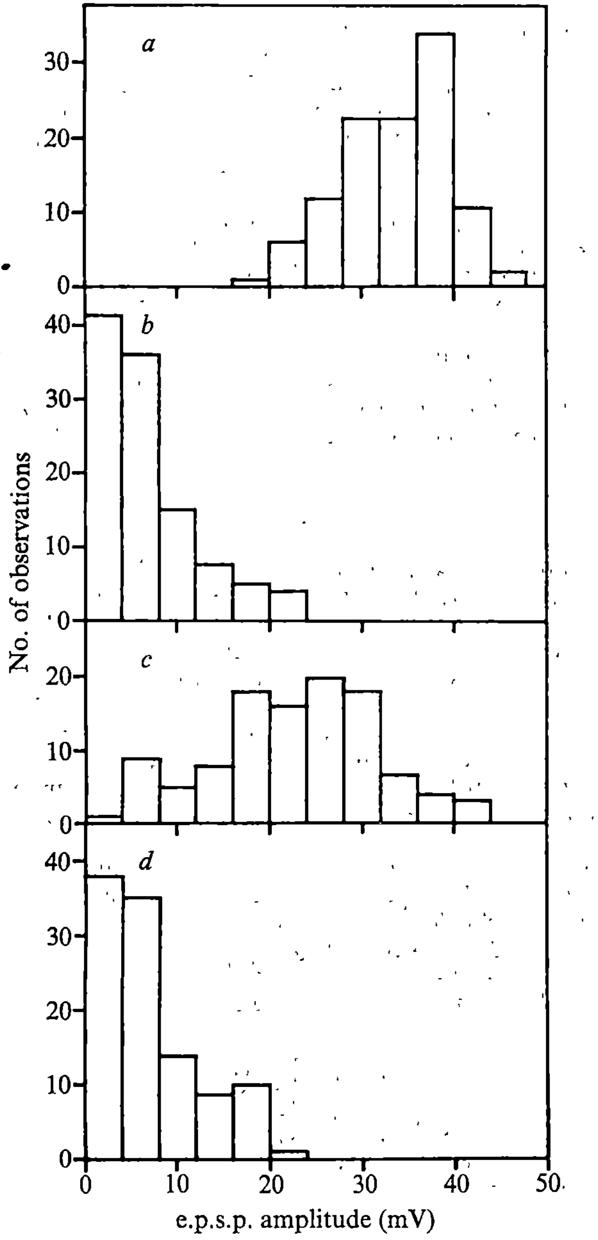


Fig. 2 Ability of exogenous NGF to curtail synaptic depression after axotomy. See text for detailed explanation *a*, Responses of normal neurones replotted from previous work[4] (number of neurones impaled $n=112$); *b*, Axotomy+control pellet ($n=109$); *c*, axotomy+NGF pellet ($n=109$); *d*, axotomy+NGF pellet, contralateral ganglion ($n=107$).

axons had been crushed; values for these parameters were in the same range as those reported previously[4,5].

In ganglia contralateral to the site of the NGF-containing pellet, neurones whose axons emerged in the crushed inferior branch gave synaptic responses that were as depressed as those in animals without exogenous NGF (Fig. 2*d*; compare with Fig. 2*b*). This result indicates that the sparing effect of NGF is a local one.

Exogenous NGF also prevents at least one other electrophysiological sign of axotomy in sympathetic neurones, the development of regenerative responses in dendrites[4,5] (see also refs 15 and 16). Rapidly rising local responses identical to those described previously[4,5] were seen superimposed on subthreshold e.p.s.p.s in 27% of axotomised neurones impaled in ganglia treated with a silicone pellet containing no NGF, and in 28% of axotomised neurones in ganglia contralateral to those treated with exogenous NGF. Such potentials were observed in only 1 of 109 axotomised neurones impaled in the NGF-treated ganglia.

The rate of NGF release from the impregnated pellets was determined by incubation for 24 h in 1 ml of sterile saline either before implantation, or after removal from the animal. The incubation medium was then assayed by the chick dorsal root ganglion method[17-19]. Pellets tested before implantation released about 1,000 biological units (BU) per ml incubation fluid per 24 h, whereas pellets which had been implanted 4–7 d before testing released 10–100 BU per ml per 24 h (1 BU corresponds to about 10^{-2} μg). Although showing that NGF is released in relatively large amounts from the pellets, these results do not provide any information about the effective concentration of NGF in the vicinity of the neurones studied.

The ability of exogenous NGF to reduce markedly synaptic depression after axotomy provides the first example of an electrophysiological effect of NGF on mammalian sympathetic neurones. We do not yet know whether the exogenous NGF acts at receptors on the surface of the cell body or intracellularly after retrograde axonal transport from the site of injury. Our results suggest, however, that NGF may normally regulate synaptic function in mature sympathetic ganglia.

We thank R. A. Bradshaw, I.-M. Jeng and R. Silverman for advice. Supported by the National Institute for Neurological Diseases and Stroke, the Muscular Dystrophy Association of America, and a US Public Health Service International Research Fellowship to A.N.

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Received January 12; accepted February 10, 1976.

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Brain immunoreactive gonadotropin-releasing hormone in Huntington's chorea and in non-choreic subjects

THERE is evidence which suggests that the release of gonadotropin-releasing hormone (GRH) is modulated by dopaminergic neurones[1-3]. Dopamine receptors seem to be hyper-responsive in Huntington's chorea[4,5], and consequently the synthesis and secretion of GRH may be altered in this condition. For this reason we have compared the concentration of immunoreactive GRH in hypothalamic tissues taken *post mortem* from choreic and non-choreic ('control') subjects. The distribution of GRH in the human brain, and changes in concentration which occur with age have also been examined.

The collection of brain tissue was carried out at Cambridge as described previously[6]. At autopsy the median eminence was removed by making a horizontal incision at the edge of the

Table 1 Mean (± s.e.m.) concentrations of GRH (pg mg^{-1}) in median eminence and hypothalamus

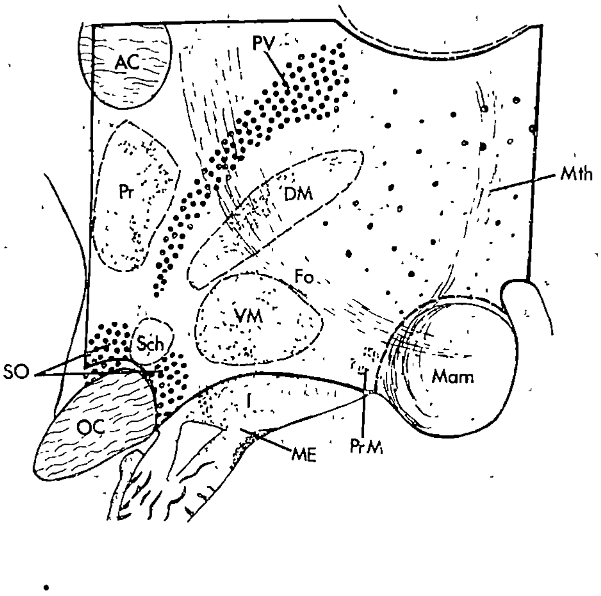
	Male		Female	
	Median eminence	Hypothalamus	Median eminence	Hypothalamus
Huntington's chorea	558±173 (6)	18 (7)	1,231±410 (9)	26 (6)
Age range	47–71	46–72	46–74	52–72
Controls	675±131 (28)	27 (23)	314±84 (11)	27 (15)
Age range	17–78	29–84	39–88	25–88

Numbers in parentheses indicate numbers of specimens. s.e.m are not given for concentrations in the hypothalamus since some values in all groups were undetectable.

vascular plexus which covers this area. The remainder of the hypothalamus (here termed 'hypothalamus') was removed as indicated in Fig. 1, the dissection being completed by vertical sagittal cuts 0.2 cm on either side of the midline. The hypothalamus of three control males (aged 39, 45 and 65 yr) was subdivided into blocks containing the preoptic, suprachiasmatic, ventromedial, dorsomedial and mammillary nuclei, respectively (Fig. 1). Frontal cerebral cortex from Brodmann's areas 9–12 of seven controls and three choreics was also taken. The tissues were stored on liquid nitrogen, and transported on dry ice to Oxford where they were weighed and homogenised (15 strokes using a Potter–Elvehjem-type homogeniser) in 0.1 N HCl at 4 °C. The homogenates were stored at –25 °C. The concentration of GRH in the homogenates was determined by a double-antibody radioimmunoassay[7,8] which is sensitive to 2–3 pg synthetic GRH per tube. The homogenates were thawed and neutralised immediately before assay, and an aliquot containing the equivalent of 0.4–0.5 mg tissue (or less at higher concentrations) was added to each of two (duplicate) assay tubes.

The slopes of the inhibition of binding curves produced by serial dilutions of the homogenates were similar to that of synthetic GRH which was used as a standard (Fig. 2), suggesting that GRH in human median eminence is immunologically similar to that found in the median eminence of the rhesus monkey, pig, sheep and rat[7,8]. Although the median eminence–pituitary stalk region had the greatest concentration of GRH, relatively large amounts of the hormone were also present in the preoptic nuclei (Fig. 1). In one specimen from a 45-yr-old male a small block of tissue was also taken from just in front of the anterior commissure. The concentration of GRH in this tissue was 55 pg mg^{-1}. The concentration of GRH in the frontal cortical tissue could not be detected (< 4.3 pg mg^{-1}) in all but one specimen from a choreic patient in which it was 16 pg mg^{-1}. In this patient, the concentration of GRH in the hypothalamus–median eminence was relatively high, 3,025 pg mg^{-1}.

Fig. 1 Diagram of sagittal section of the human hypothalamus (after Diepen[19]). The continuous lines indicate the cuts made to prepare blocks of median eminence and hypothalamus for data in Table 1. The interrupted lines indicate blocks of tissue used to determine the distribution of GRH. The mean concentrations of GRH (pg mg^{-1}) in brains from three control males were: median eminence (ME), 760±175, preoptic nuclei (Pr), 119±44; suprachiasmatic nuclei (Sch), 34±16; ventromedial nucleus (VM), 14±1; dorsomedial nucleus (DM), 12±5; mammillary bodies (Mam), 68±59. I, Infundibular nuclei; PrM, pre-mammillary nucleus; SO, supraoptic nucleus; PV, paraventricular nucleus; OC, optic chiasma; AC, anterior commissure; Fo, fornix; Mth, mamillothalamic tract.



The mean concentration of GRH in the median eminence of the female choreics was significantly greater ($P < 0.05$) than that in the female controls (Table 1). There was, however, no significant difference between the mean concentrations of GRH in the median eminence tissues from the choreic and control males. The concentration of GRH in the blocks of hypothalamic tissue was relatively small. In the control males the mean concentration (±s.e.m.) of GRH in the median eminence varied with age and was 1,315±467 ($n=6$), 535±225 ($n=5$) and 490±91 ($n=17$) pg mg^{-1} in the age ranges 17–40, 41–60 and 61–78 yr, respectively. The difference between the mean concentrations in the age groups 17–40 and 61–78 yr was significant at $P < 0.02$.

The median eminence concentrations of GRH are similar to those in rat hypothalamus (including median eminence) taken immediately before (under anaesthesia) or after death (S.A.C. and G.F., unpublished). As in the human, the greatest concentration of GRH in the rat (and guinea pig) is found in the region of the median eminence, and most but not all workers have found GRH also in the rostral hypothalamus[8–10]. The relatively high levels of GRH in the preoptic region of the human (Fig. 1) cannot be attributed to post-mortem diffusion, for, in the same specimens, the ventromedial and dorsomedial nuclei contained much smaller amounts of GRH. In the rat, the preoptic nuclei have a critical role in the causation of the preovulatory surge of luteinising hormone (LH), and may also be important for mating behaviour[11]. Preoptic GRH is conceivably present in neurones which send axons to the primary capillaries of the hypophysial portal vessels, but the presence of GRH in this region should also be considered in the context of the recent hypothesis that GRH may act as a neurotransmitter within the central nervous system[12,13].

It is not clear why the concentrations of GRH in the median eminence of choreics compared with controls differed significantly in the case of females but not males. The delay between death and the freezing of tissues was similar for the male and female specimens, and preliminary studies showed that there is no appreciable change in the concentration of GRH of hypothalamic tissue maintained at 4 °C for 6 d after death. Choreics of both sexes had been subjected to similar therapeutic regimes; all had spent the terminal stage in a psychiatric institution where they received phenothiazines regularly, and three of the males and four of the females had also been given tetrabenazine. Present radioimmunoassay methods do not enable reliable estimates of the concentration of GRH in

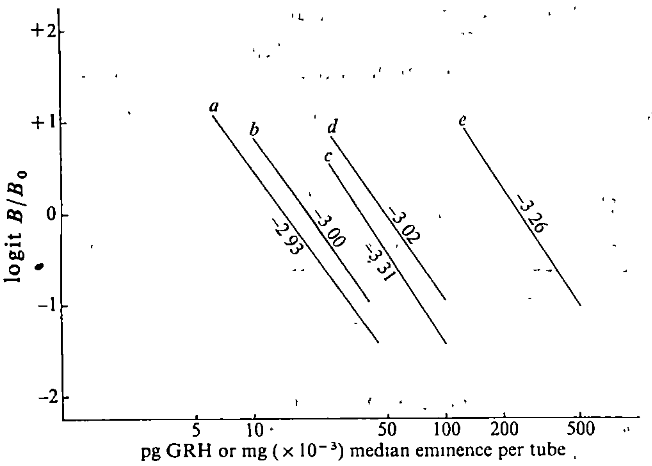


Fig. 2 Radioimmunoassay for GRH: logit* B/B_0 (percentage bound) against log dose curves of synthetic GRH (*a*) and serial dilutions of extracts of median eminence from choreic (*b*, *d*) and control (*c*, *e*) males (*c*, *d*) and females (*b*, *e*). Lines were calculated by the method of least squares from three points (four replicates per point) in the working range 20–80% bound. Numbers adjacent to the lines are the slopes. *Logit defined by ln $[y/(1-y)]$.

peripheral blood[12], and it was difficult to obtain reliable information regarding the pre-mortem menstrual or sexual history of these cases. The hypothalamic content of dopamine, noradrenaline and activity of glutamic acid decarboxylase in choreic patients is similar to that in control subjects[14].

The low concentration of GRH in the median eminence of the control females (Table 1) as well as in the older men may be due to increased release of the hormone. In the female this could not account for the marked rise in plasma gonadotropins which occurs between the ages of 45–50 yr and which is sustained at least until 75 yr (ref. 15). Although the changes are not so dramatic, the concentrations of plasma gonadotropins also increase with age in the male[15–18]. The possibility that decreased synthesis of GRH also contributes to the lower concentrations of GRH in the median eminence of the older controls cannot be excluded.

We thank the many physicians who helped us in the collection of post-mortem tissues, Miss W. McCauley-Gordon for assistance, and Dr G. D. Niswender (Colorado) and Drs H. Gregory, J. Gormley and A. L. Walpole (ICI) for gifts of R-42-anti-GRH and synthetic GRH, respectively. Supported by the National Fund for Research into Crippling Diseases (E.D.B.) and the MRC (G.F.).

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Received November 26, 1975, accepted February 12, 1976.

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Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents

IN the rat hippocampal formation, the major excitatory inputs possess a remarkable degree of physiological and morphological plasticity. Long lasting potentiation has been reported[1–3] and, at least in the dentate gyrus, the synaptic loss consequent on removal of one afferent is compensated by formation of additional synapses by intact afferents[4,5]. These phenomena must involve profound alterations of synaptic biochemistry, but the requisite biochemical studies cannot be performed until the transmitters used by these inputs are known.

One of the most critical tests of a transmitter substance is the demonstration of its release from specific fibres. Accordingly, as a first approach to the identification of the transmitters used by hippocampal afferents, we analysed the efflux from hippocampal slices of the excitatory amino acids, aspartate and glutamate. Aspartate and glutamate depolarise neurones in the central nervous system[6], and they are present in large amounts[7]. They are thus putative excitatory transmitters, although they have not been clearly related to specific fibre projections. We now report that aspartate and glutamate are released from superfused slices of hippocampal regions in a Ca^{2+}-dependent manner and that their efflux is associated in part with two excitatory afferents, the hippocampal commissural fibres and the perforant path.

In preliminary experiments, slices of rat dentate gyrus and regio superior were depolarised with 56 mM K^+ in Ca^{2+}-free medium into which Ca^{2+} was then introduced (Fig. 1). The rate of efflux of glutamate and aspartate was elevated somewhat by depolarisation of the tissue in the absence of Ca^{2+}, but the subsequent introduction of Ca^{2+} into the medium increased the efflux rate more dramatically (by 500–1,100 pmol per 30 s for a standard number of slices). This effect of Ca^{2+} was nearly always evident within 30 s. In other studies, the Ca^{2+}-evoked efflux was shown to be dependent on depolarisation of the tissue (with K^+ or veratridine); addition of Ca^{2+} to medium containing only 6 mM K^+ did not increase the rate of efflux.

Since physiological transmitter release invariably requires Ca^{2+} (ref. 8), it seemed quite possible that the Ca^{2+}-dependent portion of the depolarisation-evoked efflux was derived from synaptic boutons. In view of this relationship between Ca^{2+} and transmitter release, we tested whether boutons of the commissural or entorhinal projections to the hippocampal formation could have been responsible for the Ca^{2+}-dependent efflux of aspartate or glutamate. The commissural fibres arise from hippocampus regio inferior and terminate in all three regions of the contralateral hippocampal formation–dentate gyrus, regio inferior and regio superior[9,10]. The perforant path fibres originate in the entorhinal cortex and make synaptic contact with the granule cells of the dentate gyrus[11,12]. Some fibres of entorhinal origin also terminate in regio inferior and regio superior. In these experiments, either the contralateral hippocampal formation or both entorhinal cortices were removed, and slices of dentate gyrus and regio superior were cut, incubated and superfused as before 8–9 d after operation. We reasoned that, if aspartate or glutamate were the transmitter of the commissural or entorhinal projections, its Ca^{2+}-dependent efflux should have been reduced by eliminating the appropriate fibre tract.

By comparison with paired, sham-operated control groups, the Ca^{2+}-dependent efflux of glutamate from the dentate gyrus, but not from regio superior, was reduced almost 50% by a bilateral entorhinal lesion (Table 1). Efflux in the absence of Ca^{2+} was unaffected. Our data indicate that about half the Ca^{2+}-dependent efflux of glutamate from slices of the rat dentate gyrus is associated with the perforant path fibres, and glutamate may therefore be their transmitter.

Boutons which utilise aspartate and glutamate as transmitters are thought to possess a high affinity uptake mechanism for these amino acids[13,14]. In accordance with the postulated role for glutamate as a transmitter of the perforant path, a bilateral entorhinal lesion reduced the uptake of labelled glutamate (at a concentration below the K_m of the high affinity uptake system[13]) into slices of the dentate gyrus, but not into slices of regio superior (Table 2). In the same experiments, the high affinity uptake of GABA was unaffected by the lesion.

Commissurotomy significantly reduced the Ca^{2+}-dependent efflux of aspartate from both the dentate gyrus and regio superior (Table 1), two regions to which commissural fibres project, but did not affect efflux in the absence of Ca^{2+}. Therefore aspartate is probably released by these fibres and may be the commissural transmitter.

Commissural fibres have been shown to form additional synapses in the dentate gyrus after unilateral[15] or bilateral (unpublished) entorhinal lesions, and these connections begin to assume electrophysiological function 9 d after operation[16]. One would expect some increase in the Ca^{2+}-dependent efflux of the commissural transmitter at this time. In agreement with the hypothesis that aspartate is released from boutons of the commissural fibres, the Ca^{2+}-dependent efflux of aspartate from slices of the dentate gyrus was increased 8–9 d after a bilateral entorhinal lesion.

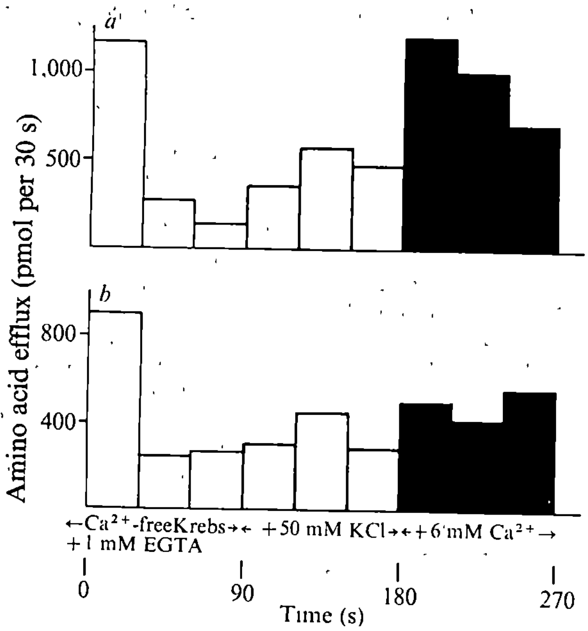

Fig. 1 Time course of glutamate (*a*) and aspartate (*b*) efflux from slices of the rat dentate gyrus Slices of about 300 μm thickness cut from 12 1-mm thick coronal slabs[18] of dentate gyrus (about 11 mg wet weight) were used in each experiment. After a 10-min incubation in Ca^{2+}-free Krebs bicarbonate medium containing 1 mM EGTA and 10 mM glucose, the slices were loaded by gentle suction on to a glass fibre filter, which was then inserted into a continuous superfusion device[19]. The slices were initially superfused at 10.8 ml min^{-1} for 90 s with Ca^{2+}-free, glucose-free, Krebs bicarbonate medium containing 1 mM EGTA. Then the K^+ concentration was increased and finally Ca^{2+} was introduced into the medium. The superfusate fractions were desalted with cation-exchange columns, and the glutamate and aspartate contents were determined by micro-enzymatic fluorometric methods[20]. The bars represent mean values for 13 (glutamate) or 7 (aspartate) experiments. Similar results were obtained with slices of regio superior.

Table 1 Ca^{2+}-stimulated efflux after lesions of hippocampal afferents

	Control (maximal increase over baseline in pmol per 30 s*)	Lesion (% of control)
Commissurotomy		
Aspartate		
Dentate gyrus	690±180	44±19(6)†
Regio superior	940±270	56±11(5)‡
Glutamate		
Dentate gyrus	710±210	91±37(5)
Regio superior	820±150	64±9(5)‡
Bilateral entorhinal lesion		
Aspartate		
Dentate gyrus	810±260	153±13(7)§
Regio superior	750±170	192±42(5)
Glutamate		
Dentate gyrus	1080±180	54±11(7)§
Regio superior	1130±360	85±19(4)

Slices were cut from 12 1-mm thick coronal slabs of hippocampal formation and superfused (see Fig. 1). Slices from operated and control animals were used in consecutive experiments conducted in random order on the same day Controls received sham lesions. Values are expressed as means±s.e.m for the number of experiments in parentheses.

*The amount of Ca^{2+}-dependent efflux was calculated by subtracting the efflux rate immediately preceding the Ca^{2+} stimulus (previously shown to remain approximately constant, if Ca^{2+} was not introduced) from the peak efflux rate during stimulation with Ca^{2+}.

Results differed from control at† $P < 0.05$, ‡$P < 0.02$, §$P < 0.01$ (two-tailed, paired *t* test). For all other differences $P > 0.05$.

In 4 of 5 experiments, the Ca^{2+}-dependent efflux of aspartate from slices of regio superior was also increased by a bilateral entorhinal lesion. We do not know, however, whether the commissural fibres form additional synapses in regio superior after this lesion.

The Ca^{2+}-dependent efflux of glutamate from slices of regio superior, but not from dentate gyrus slices, was also reduced by a commissurotomy. This effect was not attributable to inadvertant section of the crossed temporo-ammonic tract, fibres of entorhinal origin which innervate the contralateral hippocampus[9,17], since a bilateral entorhinal lesion would then have produced a similar result. Possibly some of the commissural fibres which innervate regio superior release glutamate or else glutamate is released by some other crossed pathway that was damaged.

In summary, the results presented here demonstrate lesion-induced changes in Ca^{2+}-dependent excitatory amino acid efflux from slices of hippocampal regions which are selective

Table 2 Uptake of glutamate and GABA into slices after bilateral entorhinal lesion

		^{3}H-GABA (d.p.m. per 10 min $\times 10^{-6}$)	^{14}C-Glutamate (d p.m per 10 min $\times 10^{-5}$)
Dentate gyrus	Sham	5.15±0.21	3.77±0.30
	Lesion	5.20±0.42	2.96±0.25*
Regio superior	Sham	4.20±0.60	5.05±0.98
	Lesion	4.38±0.36	4.68±0.37
		(5)	(6)

Immediately before the preliminary 10-min incubation of slices from rats with bilateral entorhinal lesions (see Fig 1), 2,3-^{3}H-GABA (10 μCi, 36.7 Ci mmol^{-1}) and U-^{14}C-glutamate (1 μCi, 237 mCi mmol^{-1}) were added at a final concentration of 0.03 μM and 0.4 μM respectively The amount of radioactivity taken up and retained by the tissue was calculated by adding the radioactivity released during superfusion to that still present in the tissue after superfusion was terminated Values are expressed as means ± s.e.m. for the number of experiments in parentheses.

*Slices from animals with bilateral entorhinal lesions differed from control at $P < 0.05$ (two-tailed, paired *t* test).

for the type of lesion, the hippocampal region and the particular amino acid. On this basis, we suggest aspartate as a strong candidate for transmitter of the hippocampal commissural fibres and glutamate for transmitter of the perforant path fibres. The possibility has not, however, been excluded that the lesions induce specific reductions in amino acid efflux from undamaged cellular elements or that substances other than those tested are also released by the fibre tracts in question. In any case, these studies should aid our understanding of adaptive mechanisms within hippocampal afferents.

We thank Miss H. Hyatt for performing the lesions and the National Institutes of Health for support.

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γ-Aminobutyric acid in rat superior cervical ganglion

RADIOLABELLED γ-aminobutyric acid (GABA) is taken up by both neurones and glial cells in the central nervous system[1,2], where it mixes with endogenous GABA stores[3]. Superior cervical and dorsal root ganglia also accumulate radioactive GABA and this amino acid is taken up exclusively in satellite glial cells[4,5]. The presence of GABA in peripheral neural tissues has not, however, been demonstrated unequivocally[6–8]. We have measured GABA and glutamic acid in rat superior cervical and nodose ganglia by mass fragmentography. The concentration of GABA in nodose ganglia was lower than in the superior cervical sympathetic ganglion (SCG). Moreover, our results suggest that the content of GABA in the SCG may be controlled by neuronal activity.

Sprague–Dawley rats (Zivic Miller) were killed by exposure of their heads and necks to a focused microwave beam for 2.2 s (ref. 9) before the dissection of SCG and nodose ganglia. In other experiments, where the effect of preganglionic stimulation on the GABA content of SCG was studied, the rats were not killed by microwave (see below). Glutamic acid and GABA were determined according to the method of Bertilsson and Costa[10]. Briefly, tissues were homogenised in 150 µl of 80% aqueous ethanol containing 330 pmol of δ-aminovaleric acid and 7.5 nmol of pentadeuterium-labelled glutamic acid used as internal standards to quantitate GABA and glutamic acid, respectively. After centrifugation, the supernatant was evaporated to dryness and the residue was reacted with 50 µl of 1,1,1,3,3,3-hexafluoroisopropanol and 100 µl of pentafluoropropionic anhydride at 60 °C for 1 h, in a small sealed vial. The pentafluoropropionyl amide hexafluoroisopropyl ester of GABA, glutamic acid and their internal standards are formed during this reaction. The reagent was evaporated and the residue was dissolved in 10 µl of ethyl acetate. About 3 µl of the solution was injected into a gas chromatograph–mass spectrometer (LKB model 9000 with a multiple ion detector). The temperature of the column (1.5 m × 3 mm internal diameter packed with 3% OV-17 on Gas Chrom Q) was 112 °C. The ionising potential was 80 eV and the trap current was 60 µA. To ascertain absolute specificity of the analysis, the intensity of three characteristic fragments of GABA (*m*/*e* 176, 190, and 204) and two of glumatic acid (*m*/*e* 202 and 230) were monitored in each determination. The mean of the relative intensity of these fragments from the SCG deviated less than 2% from those of standards. The fragments *m*/*e* 204 and 206 were used to monitor the internal standards of GABA and glutamic acid, respectively.

The GABA content of SCG was 297±18 pmol per mg protein (Table 1). Although this concentration is only a few per cent of that in brain, it is of the same order of magnitude as dopamine and noradrenaline content of SCG[11]. The GABA concentration of nodose ganglion, which has only a few synapses, was about 106 pmol per mg protein. At this low concentration, no absolute identification (by the three fragments *m*/*e* 176, 190, and 204) could be obtained; however, it can be stated that the GABA concentration in SCG is at least 3 times greater than in the nodose ganglion. The concentration of glutamic acid in the SCG (Table 1) agrees with that reported by Nagata *et al.*[6].

To determine if GABA stored in SCG is in a dynamic equilibrium the GABA content in SCG was measured 4 h after amino-oxyacetic acid injections (50 mg kg^{-1} of the hemihydrochloride intraperitoneally). Amino-oxyacetic acid, an inhibitor of GABA transaminase, the enzyme responsible for the catabolism of GABA[12], increased the GABA content of SCG more than fourfold (Table 1). Chlorisondamine HCl (10 mg kg^{-1} intraperitoneally) given 8 h before killing, SCG decentralisation (7 d before) and urethane (1,500 mg kg^{-1} intraperitoneally, 20 min before) decreased the GABA content of SCG by about 50% (Table 1). Amino-oxyacetic acid, decentralisation or urethane did not change the glutamic acid level in SCG. Thus, the GABA level was decreased by (1) blocking the ganglionic nicotine receptors, (2) destroying the preganglionic nerve terminals and (3) giving an anaesthetic agent that may act by decreasing the stimuli originating from the central nervous system and reaching the SCG[13]. These results indicate that GABA in SCG may be under neuronal control. The observation that only 50% of the GABA content disappeared after decentralisation indicates that the remaining GABA may either

Table 1 Concentration of GABA and glutamic acid in rat superior cervical ganglion

Treatment	GABA pmol per mg protein	Glutamic acid nmol per mg protein
Saline	297±18 (16)	27.5±2.6 (4)
Amino-oxyacetic acid (50 mg kg^{-1}; intraperitoneally; 4 h)	1,348±147 (4)†	36.0±3.6 (4)
Chlorisondamine (10 mg kg^{-1}; intraperitoneally; 8 h)	143±11 (4)†	NA
Decentralised	137±11 (8)†	31.1±0.9 (4)
Urethane (1,500 mg kg^{-1}; intraperitoneally; 20 min)	154±16 (4)*	30.0±3.7 (4)

Animals were killed by focused microwave beam. Values are mean±s.e.m. Number of determinations in parentheses.
* $P<0.01$; † $P<0.001$; NA, not assayed.

reflect the low steady state in absence of stimulation or originate from other sources than local synthesis; perhaps, it may be blood borne to the ganglion, where it is taken up by the glial cells.

In other experiments, rats were anaesthetised with urethane (1,500 mg kg^{-1} intraperitoneally) and 10–20 min thereafter; both SCG were exposed. One side was stimulated preganglionically at 20 Hz for 60 s (3.5 V, 0.3 ms duration pulse) and the other ganglion was used as a control. Immediately after stimulation, both ganglia (stimulated and unstimulated) were dissected out and immediately frozen on dry ice. The stimulated SCG contained more GABA (1,486±73 pmol per mg protein; $P<0.01$ by paired t test) than the unstimulated ganglia (1,256±95 pmol per mg protein). The interpretation of this result is difficult, since in the absence of microwave inactivation of enzymes the process of dissection and isolation by itself increases the concentration of GABA by about 8 times (from 154±16 in urethane-treated and microwaved animals to 1,256±95 pmol GABA per mg protein obtained in this experiment). Nevertheless, these data lend support to the hypothesis that GABA stores in SCG are under the control of neuronal activity.

We have demonstrated that the GABA content in the rat SCG is higher than in the nodose ganglion. Since the nodose ganglion has only a few interneurones and synapses, it may be surmised that the GABA storage in nodose and SCG resides in glial cells and can be regulated by the synaptic activity of the tissue. The endogenous GABA present in the glial cells of the SCG is stored against a concentration gradient by the high affinity uptake system which has been demonstrated in SCG[4]. As this GABA seems to be under control of neuronal activity, there may be a functional role of GABA in glial cells. Perhaps, it is released by the high potassium concentration which may be formed in the interstitial fluid during intense neuronal activity. The presence of neurones in the SCG that lack a GABA uptake mechanism but can synthesise GABA cannot, however, be excluded.

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Stimulation-dependent release of ^{3}H-adenosine derivatives from central axon terminals to target neurones

ADENOSINE is postulated to function in the brain as an intercellularly active neurohumoral substance. This has been suggested by *in vitro* studies showing that adenosine can be released from brain tissue by electrical stimulation at quantities which are sufficient to increase the formation of cyclic AMP[1]. The finding that adenosine derivatives are released from dendrites and particularly axon terminals points to the neurones as a major source for the released nucleotides[2]. Thus, nucleotides may function as 'secondary' or 'additional' neuronal transmitters mediating long lasting effects of a neurone on the metabolism and function of its target cells. Further evaluation of these hypotheses will require demonstrations that endogenous adenosine or its derivatives are actually released by synaptic connections in the intact brain and that this process is influenced by activation of these connections. We have carried out experiments in which tritiated adenosine was transported from cell bodies into a population of terminals, and in which the amount of radioactive materials taken up by the target cells of these terminals was measured in the presence and absence of synaptic stimulation. Stimulation was found to increase the release of ^{3}H-adenosine derivatives from central axon terminals to their postsynaptic neurones.

These studies were carried out in rat entorhinal cortex–dentate gyrus projection ('perforant path'). This system was selected because its terminals form a compact, discrete layer in the distal dendrites of the dentate gyrus granule cells. As a result of this arrangement it is possible to microdissect samples of the dentate gyrus which are purely postsynaptic to the entorhinal terminals (that is, the cell bodies of the granule cells) and to compare it with a sample which contains both terminals and postsynaptic (that is, dendritic) elements (see Fig. 1). Finally, because of its laminated organisation, the entorhinal dentate gyrus connection produces easily recordable extracellular physiological responses when activated[3]; this makes it a simple matter to establish optimal location for the electrical stimulation required in the following experiments.

Adult Sprague–Dawley rats (350–400 g body weight) while under Nembutal anaesthesia were stereotaxically injected with ^{3}H-adenosine (25 μCi = 2.3 nmol in 0.5 μl, specific activity: 11 Ci mmol^{-1}) into the dorsal aspect of the entorhinal cortex. The amounts injected were in the physiological range of the normal tissue content of adenine derivatives (2–3 nmol per mg tissue). Using recordings of the extracellular field potentials from the granule cell layer as a guide, a bipolar stimulation electrode was localised to a position in the injection site where it optimally activated the rostral dentate gyrus by way of the

Fig. 1 *a*, Region of perforant path (PP) termination (shaded area) in the hippocampus ipsilateral to the injected entorhinal cortex (EC). Positions of recording (R) and stimulating (S) electrodes as indicated. *b*, Areas sampled in the microdissection procedure (dotted area). M, Molecular layer receiving afferents from the perforant path; G, granule cell layer containing only postsynaptic elements.

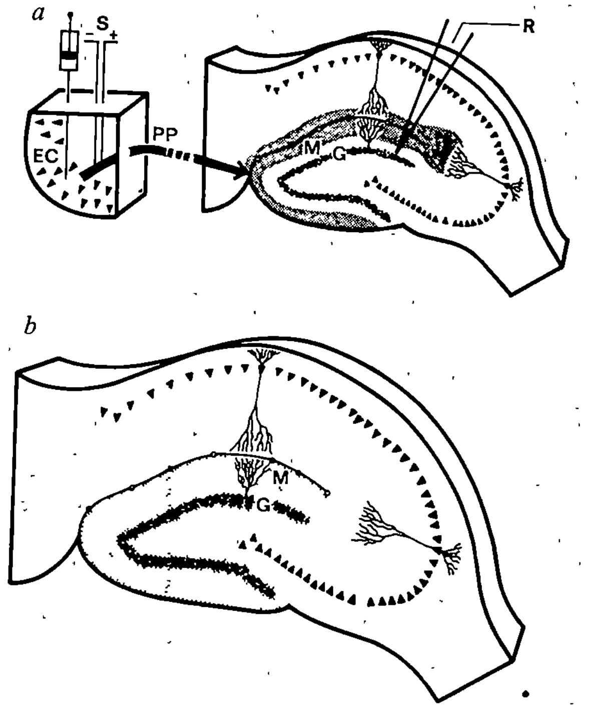

Table 1 Effect of electrical stimulation on release of ^{3}H-adenosine derivatives from axon terminals to granule cells

Duration of experiments	Ratio $G/(M+G)$ Control	Ratio $G/(M+G)$ Stimulated	% Increase in stimulated compared with control rats
5–8 h	0.20±0.03* (n = 5)	0.28±0.03 (n = 2)	40
18 h	0.19±0 03 (n = 4)	0.26±0.01 (n = 5)	37
1 d	0.26±0.04 (n = 3)	0.36 (n = 1)	39
3 d	0.33±0.01 (n = 3)	0.36 (n = 1)	9

G and *M* represent measured content of radioactivity (d.p.m.) in granule cell layer (*G*) and molecular layer (*M*) samples. ($M+G$) was usually equivalent to 8,000–14,000 d.p.m at transport times of 18 h and longer. Values are means ±s.d. *n*, Number of experiments (rats).

perforant path (see Fig. 1). Both the recording and stimulating electrodes were then cemented in place. The animal was then attached to a cable and placed in a chamber which allowed continuous recording and stimulation while the animal was awake and freely moving. Stimulation of the entorhinal cortex was carried out throughout the experiment. Pulses of 0.10 ms and 4–8 Hz were presented in a cyclic manner (10 min on and 2 min off). Such stimulation was capable of, but did not normally produce, post-tetanic potentiation of the granule cell response at the stimulus intensities used in the experiment. A second group of rats serving as controls were treated and analysed in the same way but received no electrical stimulation. The rats were killed by decapitation at various times after injection and the different samples indicated in Fig. 1 were dissected from 400-μm thick sections of the ipsilateral hippocampal target area. Homologous samples from ten slices were pooled, homogenised, sonified, and their radioactivity measured by scintillation counting. The consistency of the microdissection procedure was checked by measuring the protein content of the samples with the Lowry technique.

In normally behaving, unstimulated rats, the quantities of ^{3}H-adenosine derivatives released from the entorhinal axon terminals and taken up by the different target neurones in the hippocampus, were found to depend on the density of the entorhinal input to these neurones and the time allowed for transport (our unpublished results). The effect of electrical stimulation is shown in Table 1. For these experiments a period of 18 h was used since, at this time, a high proportion of TCA-soluble ^{3}H-adenosine derivatives had reached the ipsilateral hippocampus by way of axonal transport. Their concentration at this time was 30 times greater than that of the non-target cortical control tissue. The amount of the transported ^{3}H-nucleotides channelled to the granule cell target area (including the terminals of the entorhinal axons) is represented by the total radioactivity recovered from the molecular layer (*M*) and granule cell layer (*G*) samples. The ratio $G/(M+G)$ gives the relative content of radioactivity in the somata of the granule cells. This value will be an underestimation of the radioactivity in the granule cells since it does not include material in the distal dendrites of these cells. But this ratio represents a useful relative measure for the release to and uptake by the granule cells.

Comparison of stimulated with non-stimulated animals revealed that release and uptake were increased markedly (by about 40%) as a function of electrical stimulation (see Table 1). These results complement the *in vitro* findings of McIlwain[1]. They indicate that a functionally dependent release of adenine derivatives (which is well documented for peripheral monaminergic[4], cholinergic[5,6] and purinergic nerves[7]) also occurs from central neuronal pathways. Since the nucleotide levels in the central nervous system are rather shielded from foreign influences by the blood–brain barrier[8] they seem to be largely determined by intracerebral recycling processes. A release from axon terminals may therefore significantly alter the local concentration of adenosine at the target neurones and induce metabolic changes which reflect the amount of input received. It is tempting to speculate whether the unusual physiological plasticity of the entorhinal–dentate gyrus synapses, which seem to be potentiated for hours and perhaps days[9] after repeated stimulation, is related to adenosine-induced metabolic changes in the granule cells.

Supported by the National Institute of Neurological Diseases and Stroke.

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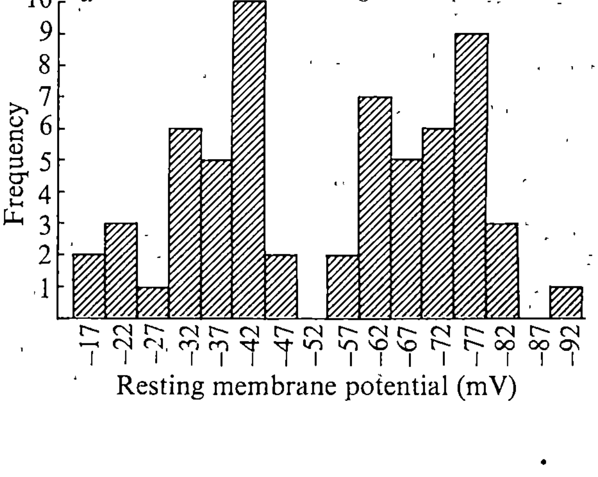
Membrane potential of juxtaglomerular cells

THE juxtaglomerular granular cell of the kidney is the site of renin storage and release, and is located most commonly within the media of the afferent arteriole, often in close apposition to the distal tubule and to sympathetic nerve endings. Morphologically, it seems to be a smooth muscle cell that has been modified for secretory activity, and contains both myofibrils and membrane-limited renin granules[1–3]. Evidence has accumulated to suggest that the juxtaglomerular cell, either directly or by way of its neighbouring cells, is stimulated to release renin by catecholamines, by changes in intravascular or interstitial pressures, and by variation in local ionic concentrations[4]. But, even though the juxtaglomerular cell is central to all theories of renin secretion, little effort has been made to investigate its physiology at the cellular level. In this paper, a new method is described for visualising living juxtaglomerular cells, and for recording their electrical activity

Fig. 1 Distribution of resting potentials of juxtaglomerular cells, showing the two groups. *a*, Group I; *b*, group II.



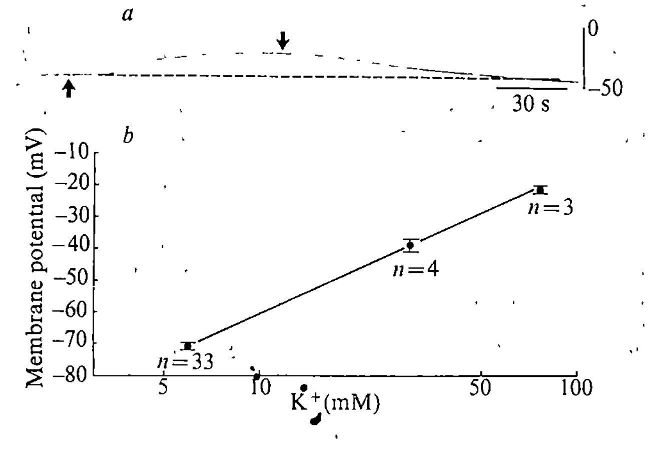


Fig. 2 *a*, Depolarisation by 70 mM K+ (first arrow) and repolarisation after return to Ringer's solution (second arrow). Ringer's solution contained (mM): Na^+ 138.0, K^+ 5.8, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 123.6, $SO_4{}^{2-}$ 1.2, $HCO_3{}^-$ 25, $H_2PO_4{}^-$ 1.2, glucose 10; and was bubbled with 95% O_2/5% CO_2. Solutions of increasing K^+ concentration were made by equimolar substitution of KCl for NaCl. *b*, Relationship of membrane potential to log $(K^+)_o$.

using intracellular microelectrodes. The results suggest that changes in the membrane potential of the juxtaglomerular cell occur with changes in renin secretion.

At least one stable resting potential was recorded in each of 25 successful experiments. For each experiment both kidneys were removed from a sodium-depleted mouse under ether anaesthesia, sectioned as thinly as possible using a razor blade, and then pressed through a 240-μm sieve. The fragments were stained with neutral red (which stains juxtaglomerular cell granules[2]), and transferred to a chamber maintained at 37 °C with Ringer's solution flowing through at a rate of 3 ml min^{-1}. Adherence of the fragments to the chamber was achieved by precoating the glass with polylysine (10 mg ml^{-1}, Sigma), a polycationic molecule that fixes tissue to glass electrostatically[5]. Glass microelectrodes, pulled with a fibre inside to tips < 1 μm, were filled by injection with 2 M KCl; tip potentials were < 5 mV, resistances 80–160 MΩ. Using Normarski optics and a ×40 water immersion lens, the juxtaglomerular cells, with their granules stained red, were readily identifiable as chains or clusters clearly distinct from the larger, diffusely stained tubular cells. Impalement by the microelectrodes proved difficult because of the small size and adventitial coats of the juxtaglomerular cells, so that it was only possible to penetrate and record stable potentials from 62 of the more than 1,000 cells in which it was attempted. In general, the potentials were stable for 10–20 s; but in several instances stable potentials were observed for up to 7 min. In those trials in which the membrane potential of a cell remained stable to within 1 mV for at least 7 s, the composition of the surrounding medium was altered by changing either the ionic composition of the flowing Ringer's solution, or by injecting drugs directly into the bath. The juxtaglomerular cells remained viable, as manifested by their exclusion of Trypan blue, for several hours. In contrast, within 1 h the tubular cells generally failed to exclude the dye.

As shown in Fig. 1, the stable resting potentials fell into two distinct populations: a low potential group, with mean −35 mV, and a higher potential group, with mean −70 mV. And, indeed, using a χ^2 test, the data were well approximated using two curves with means -35 ± 8.5 mV (s.d.) and -70 ± 7.9 mV, respectively ($\chi^2 = 15.08$, d.f. = 10, $P > 0.10$); in contrast, the data were poorly fitted by a single curve with mean -54 ± 19.7 mV ($\chi^2 = 37.36$, d.f. = 15, $P < 0.005$). It is possible that the cells with lower potentials represent a less viable group rather than a physiologically distinct population. No obvious morphological differences, however, could be found between the cells, and the whole range of potentials shown in Fig. 1 could be recorded from different cells prepared at the same time.

Since renin secretion is inhibited by increasing K^+ concentrations[6,7], it was of interest to determine whether K^+ had a significant role in determining the membrane potential of the juxtaglomerular cells. All cells that were tested ($n = 11$) depolarised with increasing K^+. Figure 2*a* illustrates the typical response to increased K^+ concentration, and shows depolarisation with 70 mM K^+ and repolarisation when normal Ringer's solution was restored in the bath. The potential was related to K^+ concentration: for cells of group I the relationship was curvilinear, as might be expected from their lower resting potentials[8], whereas for group II cells the relationship was linear (Fig. 2*b*). The slope, 43 mV per tenfold change in K^+ concentration was greater than that for many non-excitable cells[8], but less than if the juxtaglomerular cell were simply a K^+ electrode—that is

$$E_m = 61\ \text{mV}\ \log[K^+]_o/[K^+]_i.$$

Adrenaline has been shown to stimulate the secretion of renin *in vivo*[4] and *in vitro*[9,10]. As shown in Fig. 3, adrenaline caused a small, but statistically significant ($P < 0.005$) hyperpolarisation for cells over the whole range of resting potentials. Since the typical potential change with any mechanical disturbance was depolarisation, it was unlikely that these changes were artefacts. To determine the possibility of an artefact, injections of Ringer's solution into the bath were carried out for six cells. These injections caused either no change or a depolarisation, in sharp contrast to the adrenaline-induced hyperpolarisation ($P < 0.001$). A concentration of 10^{-4} M was used because of its rapid dilution by the flowing bath, and because concentrations of at least this magnitude are needed to stimulate renin secretion *in vitro*[9,10].

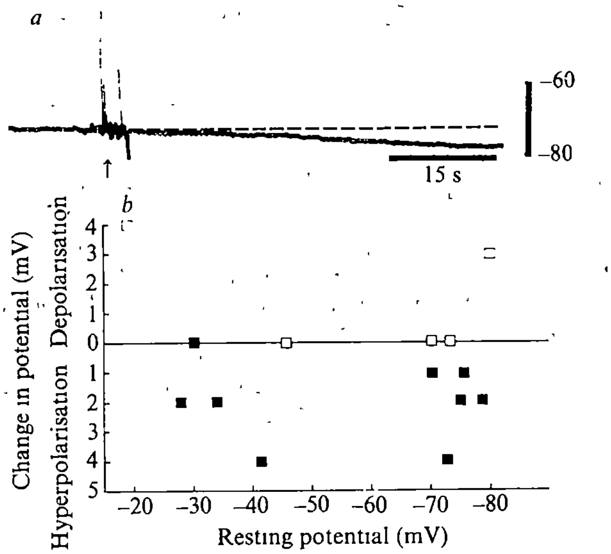


Fig. 3 *a*, Hyperpolarisation of a juxtaglomerular cell by injection (at arrow and artefact) of adrenaline (to an initial bath concentration of 10^{-4} M) into the flowing bath. *b*, Comparison of effects of adrenaline (■) or equal control (□) volume of Ringer's, for cells over the range of resting potentials.

This investigation provides a fresh approach to the study of the cellular physiology of the juxtaglomerular cell. The trials reported in this paper have demonstrated that two substances (K^+ and adrenaline) which are known to affect renin secretion in opposite ways, cause the membrane potential to change in opposite directions. These results, although distinctive, do not imply that either adrenaline or K^+ are the most important controlling elements in renin secretion, or that the membrane potential changes are the cause of subsequent alterations in renin release. Indeed, in other secretory systems, potential changes and secretion may be uncoupled[11].

Note, however, that both the adrenaline- and K^+-induced potential changes are similar to those that occur in smooth muscle cells[12], which juxtaglomerular cells resemble morphologically. One additional feature of the smooth muscle membrane potential is its sensitivity to stretch[12]. In view of the baroreceptor theory of renin secretion[13], it is tempting to postulate a similar mechanical sensitivity for the juxtaglomerular cell membrane potential. Thus, renin secretion could be partially controlled by changes in arteriolar or interstitial pressures near the juxtaglomerular cell, as signalled by changes in its membrane potential.

I thank Dr A. C. Barger for encouragement and assistance. This research was supported in large measure by the Proctor–Wellington Fund and a grant from the USPHS.

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Received January 19; accepted February 6, 1976.

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19-Hydroxyprostaglandin E_1 as a major component of the semen of primates

As their name implies, prostaglandins were first identified in secretions of the male reproductive tract. In the early 1930s several workers[1–3] showed that human semen could stimulate various smooth muscle preparations, and this activity was ascribed to an unidentified component which von Euler named 'prostaglandin'. von Euler[4,5] then found that a watery alcoholic extract of rhesus monkey seminal vesicles markedly lowered the blood pressure in the rabbit but, unlike human seminal vesicle extracts, it had little or no action on the isolated intestine and the uterus of the rat and other laboratory animals. He therefore postulated the existence of another substance in the monkey which he named 'vesiglandin' and which he suggested could be a component of human seminal 'prostaglandin'.

We have shown[6] that the major prostaglandins present in human semen are two hitherto unidentified compounds, 19-hydroxyprostaglandin E_1 (19-OHPGE$_1$) and 19-hydroxyprostaglandin E_2 (19-OHPGE$_2$), and this has since been confirmed[7]. Human semen also contains appreciable amounts of prostaglandins E_1 (PGE$_1$) and E_2 (ref. 8) which are known to both lower blood pressure and stimulate smooth muscle in test animals. It was therefore interesting to know whether these new 19-OHPGs had any pharmacological activity, and how widely they were distributed throughout the animal kingdom.

Ejaculates were obtained from men, the great apes and various monkeys and domestic and laboratory animals, either by electroejaculation[9], masturbation, or the use of an artificial vagina, and examined for the presence of 19-OHPGE$_1$ and 19-OHPGE$_2$ by gas chromatography–mass spectrometry for identification and gas chromatography alone for quantitation[10]. Table 1 shows that large amounts of these two new PGs were recovered from the semen of man, the chimpanzee, orang-utan, rhesus monkey and stump-tailed macaque, and a trace was obtained from a small and possibly incomplete ejaculate from a gorilla, whereas they were undetectable in the semen of domestic and laboratory animals. Figure 1 shows a gas chromatographic trace of the acidic fraction of rhesus monkey semen, in which 19-OHPGE$_1$ is obviously the main component.

We have already demonstrated that the production of these PGs in men is highly androgen dependent, since the concentrations in the ejaculate fell within 48 h of cessation of testosterone replacement therapy to hypogonadal individuals[11]. To investigate the site of origin of these compounds, we dissected out the complete male reproductive tract from a freshly killed stump-tailed macaque (*Macaca arctoides*) and analysed each of its components. There was an extremely high concentration of 19-OHPGE$_1$ and 19-OHPGE$_2$ in the large seminal vesicles (70 μg g^{-1}; combined seminal vesicle weight 73 g), but none was detectable in the testis, epididymis, vas deferens, ampulla of the vas or prostate. Thus there was sufficient 19-OHPGE$_1$ and 19-OHPGE$_2$ stored in the seminal vesicles to account for

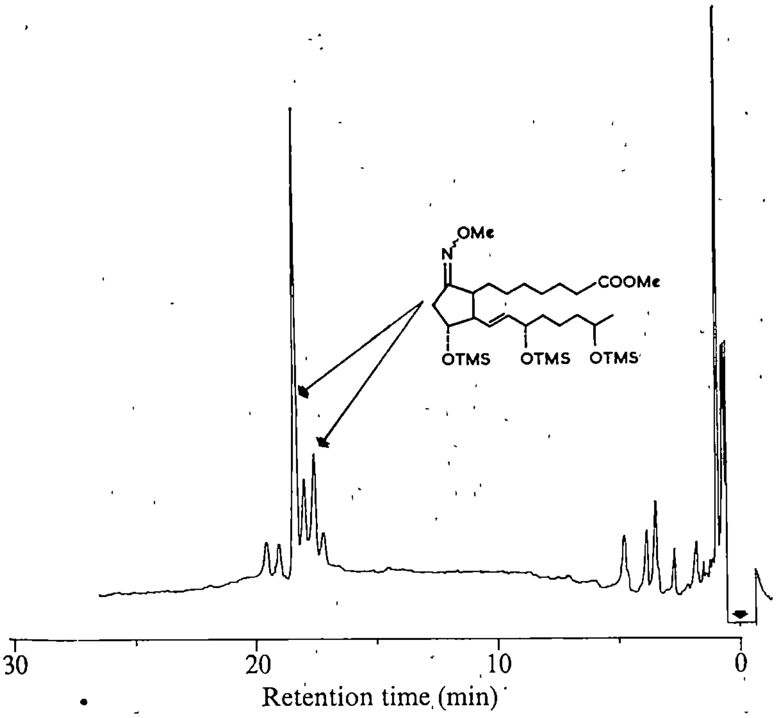

Fig. 1 Gas chromatographic (GC) trace of acidic fraction from semen of *Macaca mulatta*. Coagulated semen (2 g) was stored in methanol at −20° C, homogenised in the same methanol then centrifuged and the methanol evaporated. The residue was treated with 5 ml of a solution of methoxyamine hydrochloride (10 mg ml^{-1}) in pyridinium acetate (1.5 M *p*H 5). The mixture was left in an ultrasonic bath at 60 °C for 40 min then extracted with ether–ethylacetate (3:1); the ethereal layer was evaporated, transferred to a small tube and reacted with diazomethane followed by *bis*-(trimethylsilyl) trifluoro acetamide (20 μl). This solution was injected directly into the GC after 40 min at 60 °C. The trace was obtained from a combined gas chromatograph–mass spectrometer (duPont 490B spectrometer coupled to a Varian 1400 GC) the column was a 25-m SCOT column (SGE Ltd) temperature programmed from 170 °C to 270 °C at 4° per min. Helium carrier gas flow was 3 ml min^{-1}. Detection was by total ion current monitor.

Table 1 Concentrations of 19-OHPGE$_1$ and 19-OHPGE$_2$ in semen

Species	Source of semen	No. of samples	19-OHPGE$_1$ concentration mean (μg ml^{-1})	19-OHPGE$_2$ concentration mean (μg ml^{-1})
Man (*Homo sapiens*)	Masturbation	6	100±47 s.d.	96±43 s.d.
Chimpanzee (*Pan troglodytes*)	Masturbation	2	390	84
Gorilla (*Gorilla gorilla*)	Electroejaculation	1	5	Not identified
Orang-utan (*Pongo pygmaeus*)	Electroejaculation	2	54	Not identified
Rhesus monkey (*Macaca mulatta*)	Masturbation	8	420	84
Stump-tailed monkey (*Macaca arctoides*)	Masturbation	1	780	150
Horse (*Equus caballus*)	Artificial vagina	1	ND	ND
Cow (*Bos taurus*)	Artificial vagina	3	ND	ND
Sheep (*Ovis aries*)	Artificial vagina	8	ND	ND
Pig (*Sus scrofa*)	Artificial vagina	2	ND	ND
Rabbit (*Oryctolagus cuniculus*)	Artificial vagina	3	ND	ND
Chicken (*Gallus domesticus*)	Lumbar massage	1	ND	ND

ND Not detectable, concentration less than 1 μg ml^{-1}.

the amount present in a single ejaculate (Table 1). We examined a series of spontaneous ejaculates produced by one stump-tailed macaque that masturbated 11 times in less than 2 h (Table 2). Since the concentration of 19-OHPGEs did not decrease with time, this suggests that the vesicular store can be replenished rapidly. The high concentration of 19-OHPGE$_1$ in the semen of the stump-tailed macaque has also enabled us to isolate the compound as pure crystals (melting point 129–131 °C) by selective extraction with organic solvents, followed by Sephadex LH20 chromatography.

Dr N. S. Crossley of ICI Pharmaceuticals has synthesised 19-OHPGE$_1$ and 19-OHPGE$_2$ for us; however, these two synthetic compounds are each a mixture of the two racemic forms, [(+) and (−)], and the α and β epimers at C_{19}, whereas the naturally occurring 19-OHPGE prostaglandins are probably the (−)-racemate and the 19βOH epimer[12]. Preliminary evidence shows that synthetic 19-OHPGE$_1$ and 19-OHPGE$_2$ are about a quarter as active as PGE$_1$ and PGE$_2$ in relaxing spontaneously contracting non-pregnant human uterine muscle strips *in vitro*, whereas in contrast to PGE$_1$ and PGE$_2$, they have little or no action on rat uterine muscle *in vitro* (unpublished results of J. A. Russell, P. L. T. and R. W. K.). Since only a quarter of the synthetic material corresponds to the naturally occurring compound, it seems that the 19-OHPGEs are comparable in activity with the PGEs as far as the human uterus is concerned; moreover they are usually present in larger amounts.

Table 2 19-OHPGE concentrations in repeated ejaculates from a masturbating stump-tailed macaque (*M. arctoides*)

No.	Time	Weight of ejaculate collected* (g)	Combined concentration of 19-OHPGE$_1$ and 19-OHPGE$_2$ (μg g^{-1})
1	1100	1.71	461
2	NR	2.17	473
3	NR	0.93	439
4	NR	0.95	189
5	NR	1.07	426
6	NR	0.80	485
7	1150	0.71	496
8	NR	0.79	383
9	1205	0.73	444
10	1215	0.94	341
11	1250	0.97	433
			Total 4.96 mg

* This is a minimal estimate of the total ejaculate produced since the animal invariably ate some of it before it could be removed from the floor of the cage.

NR, Not recorded.

In contrast to human semen, with its mixture of 19-OHPGEs and PGEs, the semen from the two species of macaque contains approximately 100 times more 19-OHPGEs than PGEs. This could go some way towards explaining the different spectrum of pharmacological activity that von Euler found between human and rhesus monkey seminal vesicles.

Our study certainly emphasises the widespread occurrence of these new 19-OHPGEs throughout the primates, and the high concentrations that are present. It remains to be seen whether their potent smooth muscle relaxing activity is a significant factor in sperm transport through the human female reproductive tract.

We thank Professor T. R. R. Mann and Drs H. M. Dott, C. Polge and W. R. Allen for semen samples from domestic and laboratory animals, Dr P. E. Lake for cock semen and the Royal Zoological Society of Scotland for chimpanzee semen.

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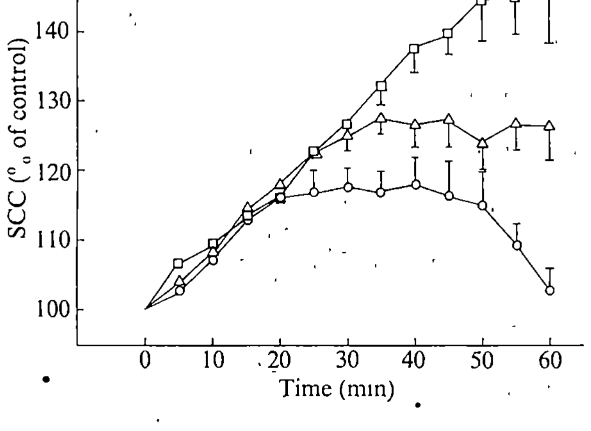
Insulin stimulates active sodium transport in toad bladder by two mechanisms

INSULIN has been shown to increase sodium and potassium fluxes in a series of tissues including muscle[1], and colon[2] and the isolated perfused kidney[3]. Attempts to define the exact mechanisms by which insulin stimulates ion transport in these tissues have been hindered by their anatomical complexity and heterogeneous cell populations. Tracer flux studies, using ^{22}Na, in frog muscle[1] and toad bladder[4] have suggested a direct stimulatory action of insulin on the sodium pump. Preincubation of intact frog muscle with insulin for 1 h has been shown to increase the amount of ouabain binding by this tissue suggesting that insulin unmasks latent sodium pumps in this structure[5]. The urinary bladder of the toad (*Bufo marinus*) has been shown to transport sodium actively from the urinary (mucosal) side to the blood (serosal) side and has been used extensively to study cation transport and the effects and mode of action of numerous hormones and drugs[6–8]. We have now examined the mechanisms by which insulin increases short circuit current (a measure of active sodium transport) in the toad bladder.

To define the effects of insulin on short circuit current (SCC), paired hemibladders were mounted in standard amphibian Ringer's in Ussing lucite chambers and the maximum SCC in response to increasing concentrations of the hormone was determined. Figure 1 shows the results of these experiments. The hormone was added to the serosal bathing media since insulin was without effect when added to the mucosal media. Concentrations of insulin greater than 50 mU ml^{-1} resulted in progressive increases in SCC. The peak response in SCC after addition of 100 mU ml^{-1} of insulin occurred at approximately 30 min and SCC returned to control values within an hour. Higher concentrations of insulin (250 and 500 mU ml^{-1}) resulted in persistently elevated SCC. An early increase in SCC was seen in the first 5 min after addition of insulin. This increase was similar but not identical to the rapid rise observed after vasopressin, a rise in Na transport which has been shown to be mediated by an increase in the cellular levels of cyclic AMP[9]. The prolonged stimulatory effect of higher concentrations of insulin on SCC was analogous to the response observed after addition of aldosterone, a phenomenon which has been shown to be dependent on protein synthesis[8,10].

Fig. 1 Effects of 100 (○), 250 (△) and 500 mU ml^{-1} (□) of insulin on short circuit current (SCC). Values are mean ± s.e. of 10 experiments.



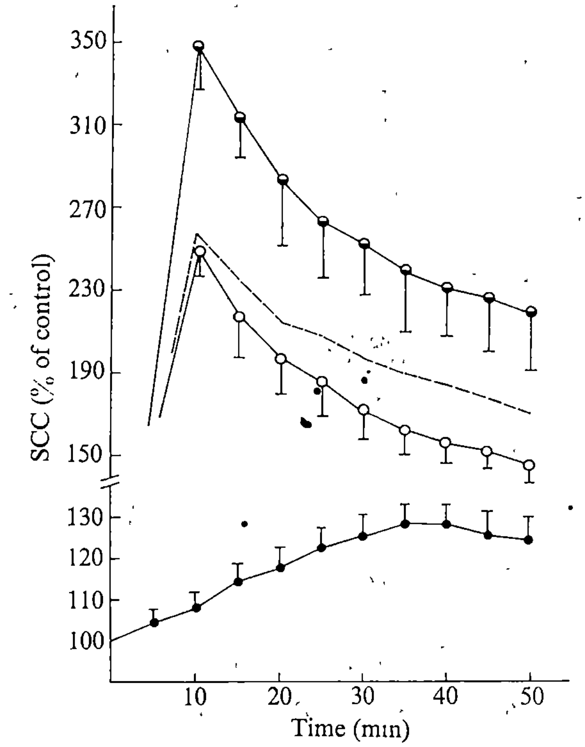


Fig. 2 Addition of 100 mU ml^{-1} of vasopressin alone (○), 250 mU ml^{-1} of insulin alone (●) or vasopressin and insulin (◒) on short circuit current. ———, Arithmetic sum of the effects of vasopressin and insulin when added alone. Values are the mean ± s.e. of 10 experiments.

We next explored the effects of addition of vasopressin and insulin alone and in combination on SCC. These results are shown in Fig. 2. Addition of vasopressin alone (100 mU ml^{-1}) resulted in a characteristic rapid increase in SCC which peaked at approximately 250% of control values. Addition of insulin alone increased SCC by approximately 30% about 35 min after addition of the hormone. When insulin and vasopressin were added simultaneously, at the same concentrations as when added alone, a marked enhanced synergistic response in SCC was observed with values rising to 340% of control. The simultaneous addition of insulin plus vasopressin thus produced an increase in SCC far exceeding the arithmetic sum of the responses observed when each hormone was added alone. In other experiments, a similar enhanced response in SCC was seen in bladders treated with insulin at time zero when vasopressin was added 40 min later. This synergistic effect was not observed, however, when the order of addition was reversed and vasopressin was added before insulin. These data suggest that insulin is capable of unmasking latent or reserve sodium pump sites not normally available to stimulation by vasopressin. An allosteric effect of membrane-bound insulin on active sodium pump sites could account for this phenomenon. An alternative explanation would require insulin to enhance or facilitate the formation of cyclic AMP in response to vasopressin, thereby acting as a modulator of vasopressin activity. To explore this possibility further, additional experiments were conducted and the results are shown in Table 1. The cyclic AMP content of scraped toad bladder cells incubated with diluent (control), insulin 250 mU ml^{-1}, vasopressin 100 mU ml^{-1} and insulin plus vasopressin were studied. Incubation time was 30 min for the experiments reported in column I. In column II the cells were preincubated with insulin for 1.5 h before adding vasopressin for the final 30 min. In neither case did insulin stimulate baseline cyclic AMP concentration nor did it increase cyclic AMP production in response to vasopressin. These data suggest that neither the early nor late stimulatory effect of insulin on SCC is mediated by cyclic AMP and that the enhanced response in SCC seen with the combination of insulin and vasopressin is not dependent on increased production of cyclic AMP. Addition of 0.5 mM cyclic AMP and insulin also resulted in a synergistic

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CALCUTTA

Table 1 Cyclic AMP levels in isolated toad bladder cells

	I	II
	(pmol per mg protein)	
Control	34.3±3.0	22.2±3 4
Insulin (250 mU ml^{-1})	35.5±5.1	23.9±4.4
Vasopressin (100 mU ml^{-1})	72.3±7.2	46.8±4.7
Insulin and vasopressin	76 1±9.1	51.5±6.1

Values are mean ± s.e. of 8 experiments.

enhanced response in SCC which was greater than the arithmetic sum of the increase in SCC observed when insulin and cyclic AMP were added alone. These data support the hypothesis that insulin may act by unmasking latent pump sites or by increasing the sensitivity of existing pump sites to cyclic AMP. Insulin does not seem to modify the binding of vasopressin to receptor sites.

To explore the role of protein synthesis *de novo* in the stimulation of SCC produced by insulin, bladders were preincubated for 1 h in Ringer containing 20 μg ml^{-1} of actinomycin D, a potent inhibitor of messenger RNA (mRNA) synthesis. Figure 3 shows the changes in SCC observed after addition of insulin to bladders incubated for the preceding 60 min with or without actinomycin D. An identical increase in SCC was observed following addition of insulin in both control and actinomycin D-treated bladders in the first 30 min. After 40 min, however, the SCC of actinomycin D-treated bladders declined and reached baseline values by 70 min. The bladders not exposed to actinomycin D showed persistent stimulation of SCC for several hours after addition of insulin.

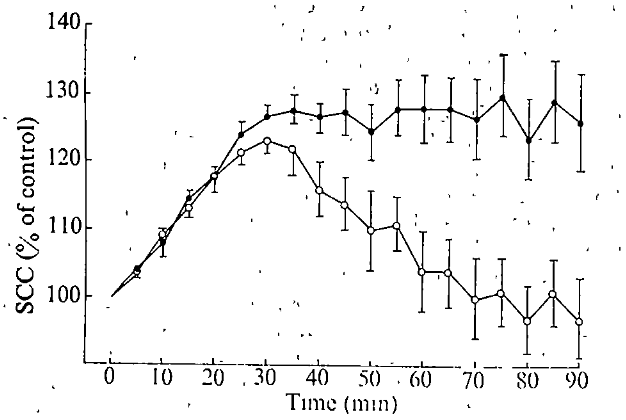

Fig. 3 Effects of addition of insulin at time 0 on the SCC of bladders incubated with (○) and without (●) actinomycin D for the preceding 60 min. Values are the mean ± s.e. of 10 experiments.

The SCC of bladders incubated with actinomycin D alone was no different from that of control bladders incubated without the drug. These data suggest that insulin stimulates active sodium transport (short circuit current) across the toad bladder by two different mechanisms. The rapid initial increase in SCC produced by insulin is unaffected by actinomycin D addition and thus does not seem to depend on protein synthesis. The rapidity of onset also speaks against the involvement of protein synthesis in this phenomenon. This initial phase of increase in SCC produced by insulin is synergistic with the effect of vasopressin, if vasopressin is added after insulin, and suggests that the initial rise in sodium transport produced by insulin may be due to unmasking of latent pump sites within the membrane. The prolonged stimulation of SCC by insulin is inhibited by preincubation of the bladders with actinomycin D. The time course of its appearance and the fact that the persistent elevation of SCC is abolished by an inhibitor of mRNA synthesis suggest that protein synthesis *de novo* is involved in this phenomenon.

It thus seems that insulin stimulates sodium transport in the toad bladder by two different mechanisms: (1) a rapid phase presumably representing "unmasking" of latent sodium pump sites within the membrane, and (2) a prolonged phase of increased sodium transport that requires synthesis *de novo* of protein. The nature and mode of action of this newly synthesised protein(s) on sodium transport are unknown and further studies will be required to elucidate its role.

This work was supported by the US Public Health Service, US National Institutes of Health. During these studies W.P.W. was the recipient of a research fellowship from the Missouri Heart Association.

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Received December 8, 1975: accepted February 6; 1976

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Diminution of tumorigenesis initiated by 4-nitroquinoline-1-oxide by post-treatment with caffeine in mice

THE potent carcinogen 4-nitroquinoline-1-oxide (4NQO)[1] mimics ultraviolet effects[2,3]. Kondo[4] proposed an idea to test the mutation theory of cancer by comparison of 4NQO carcinogenesis in mice and 4NQO[3] or ultraviolet[5] mutagenesis in *Escherichia coli*. In parallel with 4NQO mutagenesis in *E. coli*[2,3,6], it was concluded that transformation initiated by 4NQO in cultured mouse cells is suppressed by holding in a non-DNA-replicating state[7], due to elimination of 4NQO–purine adducts[8] from DNA by an error-free excision repair[9]. There is another type of repair which works after DNA replication and is assumed to be error prone[5]. Again, in parallel with ultraviolet mutagenesis in *E. coli*[10], Kakunaga[7] has found that transformation of mouse cells by 4NQO is reduced greatly by post-treatment with caffeine. Zajdela and Latarjet[11] reported that ultraviolet-inducible skin cancer in mice is suppressed by the presence of caffeine. The present paper reports that post-treatment with caffeine diminishes 4NQO-initiated lung tumorigenesis in mice.

Young (21-d-old) ICR/Jcl mice (9–11 g), 339 males and 285 females were used (Japan Central Laboratory for Experimental Animals). 4NQO (provided by the late Dr K. Mori, Showa Medical School, Tokyo) was dissolved in propylene glycol and caffeine (Nakarai Chemical Co.) in water. Both chemicals were injected subcutaneously; 4NQO in the right flank of animals with a single dose (12.5 μg per g body weight) and caffeine in the left flank with five doses (100 μg per g body weight for each) at intervals of 6–12 h (Fig. 1). These doses of 4NQO and caffeine are the maximum tolerated doses to young ICR/Jcl mice. As described in Fig. 1, animals were divided into five groups. Groups 1 and 2 received caffeine during the 0–36 h and the 120–156 h after 4NQO treatment, respectively, but group 3 received caffeine during the 12–48 h before 4NQO treatment. Group 4 received an equal volume of water, instead of caffeine solution, during the 0–36 h after 4NQO treatment. Solvent control, group 5, received an equal volume of propylene

Table 1 Effects of caffeine on 4NQO-induced tumour yields in mice

Experimental groups					No. of mice at start	Lung tumours			
No.	4NQO (μg g⁻¹)	Caffeine (μg g⁻¹)	Period (h) of caffeine treatment after 4NQO	Sex		Incidence (%)	χ^2 test*	Tumours per lung (mean±s.e.)	t test*
1	12.5	100	0–36	M	100	26/57 (45.6)	NS	1.04±0.22	NS
				F	85	24/54 (44.4)	$P < 0.05$	1.85±0.43	$P < 0.05$
2	12.5	100	120–156	M	46	8/26 (30.8)	NS	0.58±0.20	$P < 0.01$
				F	46	15/28 (53.6)	NS	1.29±0.38	$P < 0.01$
3	12.5	100	−48–−12	M	30	10/22 (45.5)	NS	1.82±0.69	NS
				F	30	17/28 (60.7)	NS	3.07±1.11	NS
4	12.5	0.0	—	M	122	41/98 (41.8)	—	1.59±0.28	—
				F	79	37/57 (64.9)	—	4.42±1.05	—
5	0.0	100	—	M	41	1/34 (3.0)†	—	0.03±0.03†	—
				F	45	3/45 (6.7)†	—	0.07±0.04†	—

*χ^2 test and t test were applied for groups 1–3 against group 4. t test was applied with the Cochran–Cox approximation, if variance ratio was more than F value at 1%. NS, Not significant.

†All 4NQO-treated groups (groups 1–4) developed lung tumours with significantly higher incidence and frequency than solvent control (group 5), ($P < 0.01$) Spontaneous tumour incidence and frequency at week 25 were 2/315 (0.6%) and 0.006±0.005, respectively.

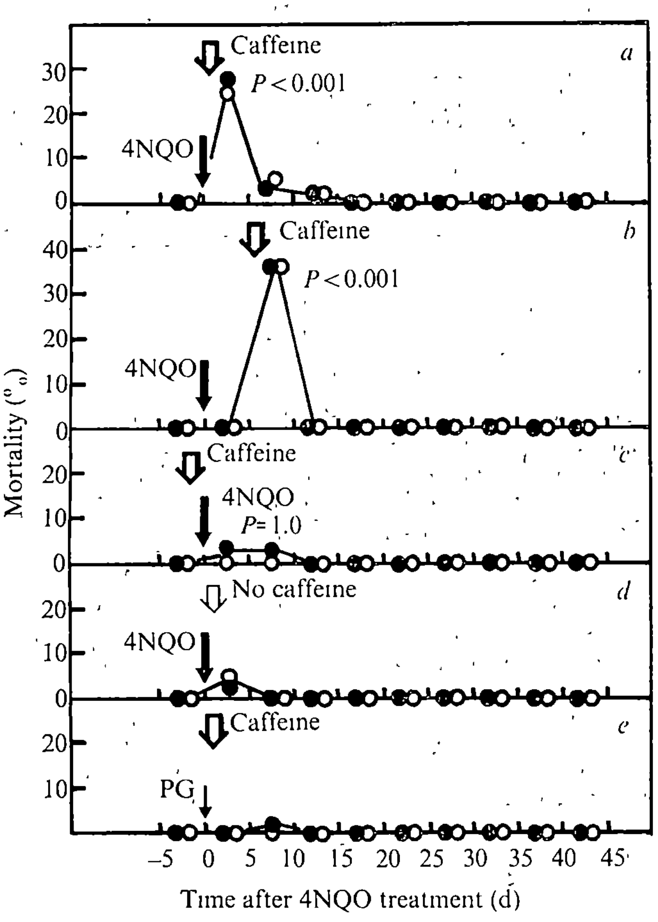

Fig. 1 Enhancement of mortality in 4NQO-treated mice by post-treatment with caffeine Percentage of dead mice (●, male; ○, female) is plotted against days after 4NQO treatment. Lethality of 4NQO-treated mice confined to 2–5 d after caffeine treatment, primarily due to intestinal death Numbers of mice used are given in Table 1 χ^2 test was applied with Yates' correction for groups 1–3 against group 4. *a*, Group 1: five subcutaneous injections of caffeine (100 μg per g body weight for each) were given to young mice 0, 6, 12, 24, and 36 h after a single injection of 4NQO at a dose of 12.5 μg per g body weight ($P < 0.001$) *b*, Group 2: five doses of caffeine were given 120, 126, 132, 144, and 156 h after 4NQO treatment ($P < 0.001$). *c*, Group 3: five doses of caffeine were given 12, 24, 36, 42, and 48 h before 4NQO treatment ($P \neq 1.0$). *d*, Group 4: an equal volume of water, instead of caffeine solution, was given after 4NQO on the same time schedule as in *a*. *e*, Group 5: propylene glycol (PG), instead of 4NQO solution, was given, and then followed by five doses of caffeine on the same time schedule as in *a*.

glycol, instead of 4NQO solution, and caffeine during the following 0–36 h. The mice were maintained on mouse diet CA-1 (CLEA)[12], and killed at week 20 after 4NQO treatment to examine lung tumours as described previously[12,13]. Most lung tumours were papillary adenomas[12,14]. Lung tumour frequencies, defined as average numbers of tumour nodules per lung, were compared statistically among the experimental groups, since the frequency thus defined increased with doses of carcinogens, even in the high dose range where the increase in the tumour incidence defined as fraction of tumour-bearing mice in survivors levelled off[12,14].

Table 1 summarises the results. It will be clear from comparison of groups 1 and 2 with group 4 that in females post-treatment with caffeine during either the early (0–36 h) or late (120–156 h) post-4NQO period suppressed tumour frequency by 60–70%, and that in males caffeine administered during the 120–156 h after 4NQO treatment significantly suppressed the tumour frequency. The tumour frequency in males post-treated with caffeine during the 0–36 h after 4NQO treatment (group 1) was also lower, but not significantly lower, than that of males treated with 4NQO alone (group 4). This will, however, not invalidate the conclusion that caffeine post-treatment suppresses 4NQO-initiated lung tumorigenesis, because the less suppressive effect of caffeine during the 0–36-h period compared with the 120–156-h post-4NQO period was also the case with females (Table 1). Furthermore, it is the rule deduced from 14 sets of our previous experiments with 50–60 young ICR/Jcl mice each, that lung tumour yields are always lower in males than in females after treatment with 4NQO, but not with other carcinogens[12]. Caffeine administered during the 12–48 h before 4NQO treatment gave no statistically significant effects on the 4NQO-induced tumour frequencies in both males and females (groups 3 and 4, Table 1). In parallel with the case of cultured mouse cells[7], post-treatment with caffeine during either the 0–36 h or the 120–156 h after 4NQO treatment, but not pre-treatment, increased mortality in 4NQO-treated mice to about 30% compared with negligible mortality in mice treated with 4NQO alone (Fig. 1). These support the misrepair model[4] in that caffeine suppresses the repairing action of error-prone post-replication repair on tumorigenic and lethal damage produced in daughter DNA by 4NQO damage on template DNA, resulting in decrease of survivors and induced tumours. The lethal and tumorigenic damage in mice is sensitive to caffeine as late as 120 h after 4NQO treatment (Table 1 and Fig. 1), but cultured cells become insensitive to caffeine as early as 48 h after 4NQO treatment[7]. This difference may simply reflect the fact that a considerable fraction of DNA, bearing 4NQO damage, in the stem cells of the lung remains unreplicated for several days, whereas almost all the cultured cells in the growing state go through the phase of DNA replication in about one day after 4NQO treatment. Thus I propose that not only 4NQO-induced pretransformational damage in cultured mouse cells but also 4NQO-induced pretumorigenic damage in mice may be fixed during the first post-4NQO DNA replication period, the fixation being similarly suppressed by caffeine in mice as well as in their cultured mouse cells. It has been reported[15,16] that actinomycin D inhibits skin tumour formation initiated by 7,12-dimethylbenz(*a*)anthracene or β propiolactone when given 0 to 14 d later, provided that actinomycin D is present, which probably works as inhibitor for normal DNA synthesis, during the first post-carcinogen DNA replication. Caffeine is known to slow down greatly the post-replication processes and to inhibit specifically post-replication repair in cultured mouse cells after treatment with ultraviolet[17] or 4NQO (M. Morita and M. Tada, personal communication).

I carried out this work as a member of a cooperative cancer research team headed by Dr S. Kondo with a grant from the Japanese Ministry of Education, Science and Culture.

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Inhibition of RNA polymerase as a possible anti-leukaemic action of cytosine arabinoside

Cytosine arabinoside (ara-C, 1-β-D-arabinosylcytosine) has marked anti-tumour and anti-viral activities[1,2], and is currently one of the most successful drugs used to induce remissions in acute granulocytic leukaemia[3]. Its cytotoxic activity is ascribed to its effect of inhibiting DNA polymerase after being phosphorylated intracellularly to its triphosphate derivative (ara-CTP)[4]. Studies with murine leukaemic cells[5] and mouse L cell line have indicated[6], however, that acute cell death induced by ara-C does not completely correlate with the inhibition of DNA synthesis: (1) DNA polymerase activity was rapidly restored after ara-C was removed, whereas cells were still non-viable; (2) the amount of ara-C incorporation into DNA did not correlate with the degree of cell lethality. We have shown previously[7] that ara-CTP could inhibit RNA polymerase II activity from chicken leukaemic cells when the enzyme was purified. This report describes results of our kinetic studies characterising the nature of this inhibition.

Chicken leukaemic RNA polymerase IIa, obtained from glycerol-gradient centrifugation as described previously, was used for the present studies. The effect of ara-CTP on the kinetics of incorporation of CTP into RNA was determined

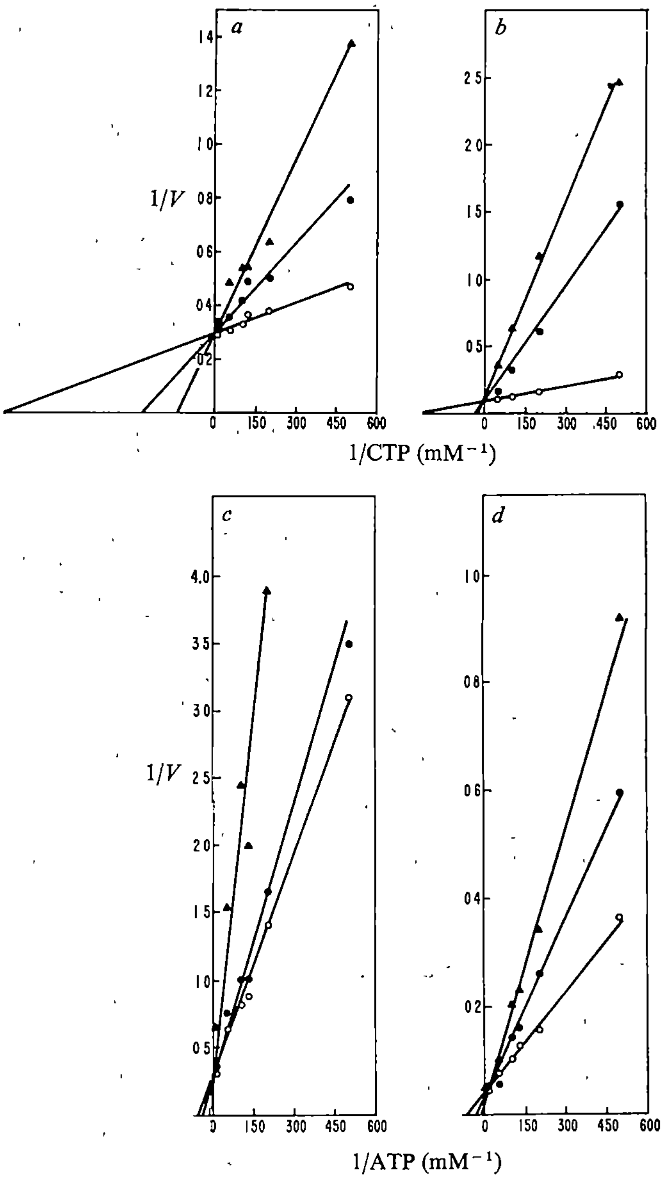

Fig. 1 Effect of ara-CTP and ara-ATP on the kinetics of RNA synthesis (Lineweaver–Burk plot). The reaction mixture (0 1 ml) for chicken leukaemic enzyme (*a* and *c*) contained 50 mM (*p*H 7.9) Tris-HCl, 2 μg pyruvate kinase, 4 mM phosphoenol pyruvate, 1 mM MnCl$_2$, 10 mM KCl, 1 mM dithiothreitol, 40 μg denatured chicken blood DNA (Calbiochem), 2 μg RNA polymerase, and four normal ribonucleoside triphosphates, with or without the addition of drug. The reaction mixture for *E. coli* enzyme (*b* and *d*) was similar to that described above except that it contained RNA polymerase (*b*, 0.25 U; *d*, 0.5 U; fraction IV, Grand Island Biological), 4 mM MgCl$_2$, 1 mM MnCl$_2$, 5 mM dithiothreitol and 40 μg native *E. coli* B DNA (Sigma). The four normal ribonucleoside triphosphates used were 0.2 mM GTP, 0.04 mM UTP and ^{3}H-UTP (1,000 c.p.m. pmol^{-1}); and 0.2 mM ATP and varied CTP concentration (*a* and *b*); 0.2 mM CTP and varied ATP concentration (*c* and *d*). Incubation was at 37 °C for 15 min. The reaction was terminated and the RNA precipitated, filtered and counted as described previously[7]. 1/*v* shown on the ordinates is the reciprocal of pmol UMP incorporated into RNA per 0.1-ml reaction mixture per 15 min. Symbols: *a* (K_i for ara-CTP = 14.9 μM): ▲, 0.1 mM ara-CTP; ●, 0.03 mM ara-CTP; *b* (K_i for ara-CTP = 10.2 μM): ▲, ●, as *a*; *c* (K_i for ara-ATP = 150 μM): ▲, 0.3 mM ara-ATP; ●, 0.1 mM ara-ATP; *d* (K_i for ara-ATP = 77.5 μM): ▲, ●, as *c*. ○, No drug added.

Table 1 Effects of arabinosyl nucleotides on RNA synthesis by chicken leukaemic RNA polymerase II

Added nucleotide	Control	ara-AMP	ara-ADP	ara-ATP	ara-CMP	ara-CDP	ara-CTP	ara-UTP
UMP incorpoated (pmol ml^{-1})	27.5	30.5	22.0	1.60	30.4	26.6	1.70	29.4
% control	100	110	80	5.9	110	96	6.3	106

Chicken leukaemic RNA polymerase II (20 μg ml^{-1}) was assayed in the presence of 1 mM arabinosyl nucleotide as indicated. The assay conditions were similar to those described in Fig. 1, except that all four normal ribonucleoside triphosphates used were 0.04 mM, and that the incubation (0.2 ml) was for 45 min. The arabinose nucleotides were obtained from Terra-Marine Bioresearch, La Jolla, California.

and the results are presented in Fig. 1*a*, using the graphical representation of Lineweaver and Burk[4]. Ara-CTP is a competitive inhibitor for the binding of CTP to RNA polymerase II; K_m (the Michaelis constant) is increased in the presence of the drug without change in V_{max} (the maximum velocity of the reaction). The K_m for CTP and the K_i (enzyme inhibitor dissociation constant) value for ara-CTP inhibition were estimated from 6 determinations to be 1.4±0.5 μM and 14.9±3.0 μM, respectively, for the leukaemic enzyme. A similar study was carried out on *Escherichia coli* RNA polymerase and it was shown that the enzyme was also inhibited by ara-CTP with a K_m (CTP) of 4.2±0.2 μM and a K_i (ara-CTP; from seven determinations) of 10.2±2.7 μM (Fig. 1*b*).

The reported K_i values for ara-CTP inhibition of DNA polymerase (replication enzyme) were 1 μM for calf thymus[4], 8.7±5.2 μM for mouse L cells[6], 18.4±7.9 μM for mouse lymphoma cells[8]. The K_i value reported for Rauscher leukaemia virus reverse transcriptase was 30 μM (ref. 9), and for DNA repair enzyme of isolated hepatocyte nuclei, 500 μM (ref. 10). Our studies with RNA polymerase showed that the K_i value for ara-CTP inhibition for chicken leukaemia enzyme fell in the same range of K_i values for the DNA replication enzyme in mouse L cells and mouse lymphoma cells, and that it was certainly smaller than the values reported for reverse transcriptase and DNA repair enzyme. It indicates comparable affinities of ara-C for the RNA polymerase and the DNA replication enzyme. Because of the competitive nature of this inhibition, the relatively high I_{50} value reported previously[7], could be explained by the high CTP concentration used in the assay system. It would seem that the degree of inhibition by ara-C of cellular DNA compared with RNA synthesis will depend on the physiological (local) concentrations of dCTP and CTP, the concentration of ara-C used, and their individual K_m values. It has been suggested[10,11] that inhibition of replicative DNA synthesis by ara-C could not kill cells and that to achieve cell killing ara-C must be given at a dose high enough to impair DNA repair function. At such a high ara-C dosage, however, inhibition of RNA synthesis has undoubtedly occurred.

The anti-tumour and anti-viral activities of adenine arabinoside (ara-A) have long been recognised[12,13], although data on clinical trials of this compound are scanty. The compound was suggested to have no effect on RNA polymerase activity[14,15], and the inhibition by ara-A of DNA polymerase was shown to be selective[13]. We tested the effects of ara-A on the purified RNA polymerase and found that it inhibited the enzymes by competing with the normal nucleotide ATP. The mean K_m (ATP) and K_i (ara-ATP) values were 17.2 and 150 μM, respectively, for chicken leukaemic enzyme, and were 14.2 and 77.5 μM, respectively, for *E. coli* enzyme (Fig. 1*c* and *d*). These K_i values are in the same range as those reported for ara-A inhibition of DNA polymerase[12].

It is well known that ara-C is rapidly deaminated to uracil arabinoside (ara-U) *in vivo*, and that the latter compound has no pharmacological value[16]. The negative effect of ara-U on tumour cells prompted us to study the effect of ara-UTP on chicken leukaemic RNA polymerase. The results are presented in Table 1. Ara-UTP did not inhibit the RNA polymerase activity, suggesting that the amino group of cytosine or adenine base may be essential for the inhibition. Further studies showed that the inhibition of RNA polymerase activity by ara-C or ara-A was at the triphosphate level of the drugs. Neither mono- nor diphosphates produced the inhibitory effect (Table 1). These findings are in accord with *in vivo* observations[11] that cellular levels of ara-CTP, but not those of the mono- and diphosphates of ara-C, are the determinants of chemotherapeutic efficacy of ara-C against leukaemia. These, together with the ara-U studies, suggest the possibility of considering RNA polymerase inhibition as the target for ara-C action.

This study has described the inhibitory activity of ara-C (or ara-A) on RNA polymerases. Since RNA polymerase II has been suggested as being responsible for messenger RNA synthesis[17], the inhibition of RNA polymerase II by ara-C may result in the decreased 2–7S RNA synthesis[18] and subsequently in an irreversible cell death, as observed previously[5,6,18]. Since ara-CTP has apparent affinity for RNA transcription comparable for DNA replication, inhibition of RNA synthesis must be considered before the mechanisms of this drug can be understood completely.

This research was supported by the National Cancer Institute and the United Health Service of North Carolina.

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Received November 21, 1975; accepted February 10, 1976.

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Similarities in the helical sequences of the repressor-binding sites in the *lac* and λ operators

SEVERAL reports have considered the repressor binding sites in the *lac*[1–3] and λ[4–6] operators. The analysis of these regions has focused on features apparent in the linear sequences such as regions of twofold rotational symmetry, and the 17-base-pair sequence which is largely conserved in the rightward and leftward repressor-binding sites of λ[6]. As these sequences are thought to be wound in the double helical form when they are recognised by repressor[7,8], I have investigated whether any further interesting features could be revealed by such an arrangement.

There are many possible ways of "casting" a sequence on a

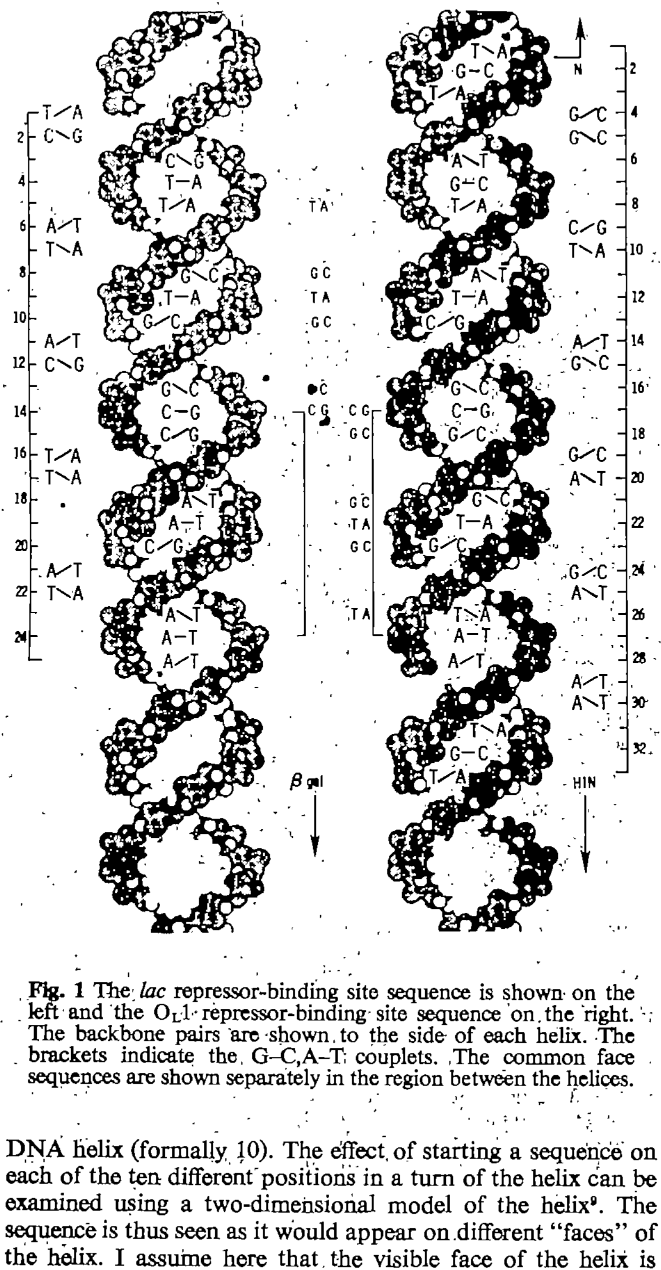

Fig. 1 The *lac* repressor-binding site sequence is shown on the left and the O_L1 repressor-binding site sequence on the right. The backbone pairs are shown to the side of each helix. The brackets indicate the G–C,A–T couplets. The common face sequences are shown separately in the region between the helices.

DNA helix (formally 10). The effect of starting a sequence on each of the ten different positions in a turn of the helix can be examined using a two-dimensional model of the helix[9]. The sequence is thus seen as it would appear on different "faces" of the helix. I assume here that the visible face of the helix is composed of the three base pairs centred in each groove. The two base pairs aligned most nearly parallel to the line of view at each half turn are assumed to be hidden behind the backbone and therefore not seen on the forward face. Thus a "face sequence" is the sequence presented by the base pairs visible in the grooves with the backbone pairs omitted. Therefore, the sequence one sees on a particular face is composed of the three base pairs in the centre of, say, a wide groove, then the three pairs in a narrow groove, then the three in a wide groove, and so on; the two base pairs between grooves are masked and not seen. For the purpose of looking for symmetries, no distinction is made here between wide and narrow grooves, so half the possible castings become equivalent.

When this procedure was applied to the binding sites of *lac*[1] and λ O_L1 (ref. 4), the result was unexpected. There was for each sequence, one "casting" which established two types of homologous features shared by the two repressor-binding sequences on one particular face of the helix (Fig. 1). These are: (1) a face sequence in each comprised of the same six "visible" base pairs with only the 5′–3′ orientation reversed, and (2) a feature which, for lack of an established terminology, will here be called a G–C,A–T couplet; this is defined simply as a succession of three G–C base pairs preceded or followed, at a separation of seven base pairs, by a succession of three A–T base pairs, with the result that one wide groove filled by G–C pairs would have an adjacent wide groove filled by A–T pairs in the helix.

As Fig. 1 shows, the 'upper' half of the *lac* sequence (positions 5–14), excluding backbone positions, is a perfect reflection of the lower part of the λ sequence (positions 17–26). As discussed below, this sequence is largely conserved in the O_R1 (ref. 5) and O_L2 (ref. 6) binding sites in that it forms part of the sequence which Maniatis *et al.* have noted to be repeated in the rightward and leftward λ repressor recognition sites. Considering *lac* and O_L1 sequences alone, the random odds of such a match symmetrically located with respect to the centre of the sequences are less than 1 in 1,000. (The probability of a random match in a sequence of size six is $\sim(1/4)^6$ since the G–C content in *E. coli* and λ DNA is 51%. There are four chances for a match in the 4 halves of the two sequences. The probability of one or more such matches is then $1-P(0)$ or $1-[1-(1/4)^6]^4 = 0.00097$.)

The second homologous feature shared by the *lac* and O_L1 sequences as presented in Fig. 1 is what I have termed a G–C, A–T couplet in each sequence. The central wide groove contains only G–C pairs and the next lower wide groove contains only A–T pairs. The random odds of such matching couplets are $\sim 2(1/64)^2$ or 1 in 2,000. This feature, as noted below, also appears in O_L2, O_R1, O_R2 and O_R3. Alignment of the *lac* and O_L1 sequences, so that this feature is seen in the forward face, defines a helix face with considerable symmetry. For example, the symmetry of the *lac* sequence is still present in this representation. Most of the symmetry properties discussed here are limited to positions 4 to 24, noted by Gilbert and Maxam[1] to show substantial rotational symmetry about position 14 when viewed in the linear form. The two asymmetries previously observed in this rotation are split between the face sequence ($10\nrightarrow18$) and the backbone sequence ($12\nrightarrow16$); otherwise both face and backbone sequences still show rotational symmetry about position 14. It is interesting that the "correction" of the $12\nrightarrow16$ asymmetry is a known constitutive mutation, and a correction of the $10\nrightarrow18$ asymmetry would cause a recurrence in the lower half of the *lac* sequence of the common sequence shared by *lac* and O_L1. On the other hand, the symmetry seen in this view of the O_L1 sequence was not suggested by Maniatis *et al.*[4] but is clearly revealed by this format. In contrast to the *lac* sequence, the symmetry character of the O_L1 sequence when viewed on this face of the helix is not a rotation but a reflection, in the face sequence, with seven of the ten face pairs reflected across the centre. This symmetry extends beyond the homologous regions picked out by Maniatis *et al.*[6]. The backbone sequence shows neither type of symmetry.

It is intriguing that this feature, the G–C,A–T couplet, appears in several other control sequences. The phage fd RNA polymerase binding site[10] has such a couplet at its centre. The CAP sequence starts with two such couplets[2]. One face in the promoter sequence of the *tyr* tRNA gene[11] shows three such couplets out of a possible four for that sequence size; in this case, however, it is a different face of the helix which shows perfect rotational symmetry centred on the rotational axis noted by Sekiya and Khorana[11] (here all asymmetries in the sequence fall on the backbone positions). The sequence for the attenuator region of the tryptophan operon[12] contains a successive series of six such G–C,A–T couplets in a row. This guarantees one such couplet on every face of the helix in the attenuator region.

In Fig. 2, the sequences of the O_L2, O_R1, O_R2, and O_R3 binding sites[5,6] have been aligned such that the G–C,A–T couplet is seen in each. The same alignment shows most of the *lac*–O_L1 common sequence appearing in O_L2 and O_R1; the one change is that T–A at position 22 in O_L1 becomes A–T in O_L2 and O_R1. This is the only change between O_L1 and O_R1 which falls within the recurring symmetrical 17-base-pair regions described by Maniatis *et al.*[6] and is viewed by them as the probable cause of the weaker binding observed with O_R1.

The fact that the G–C,A–T couplet appeared in all these sequences and in several cases was associated with a recurring sequence suggested a search of the only other sequence known

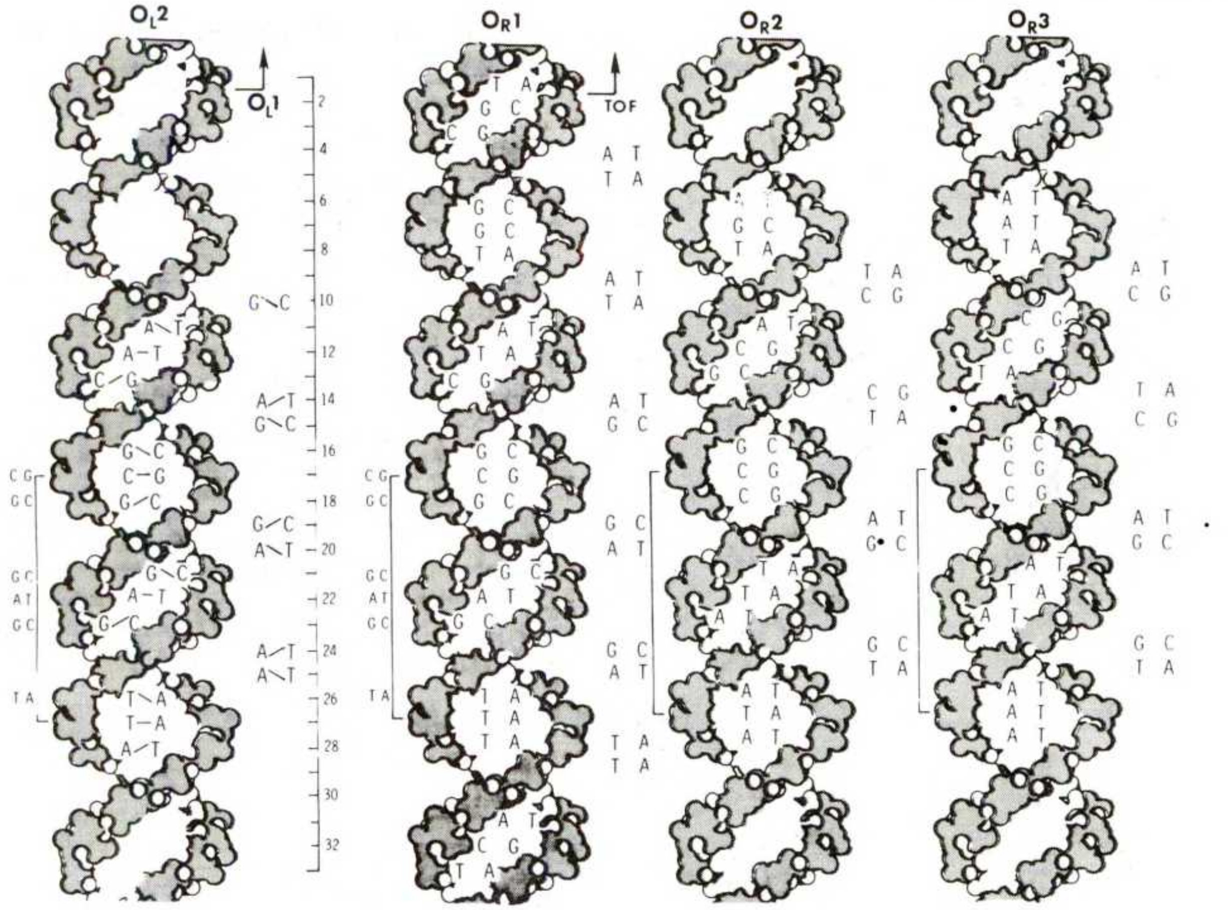

Fig. 2 The helices from left to right represent the repressor-binding sequences of O_L2, O_R1, O_R2 and O_R3, respectively. The common face sequence shared by O_L1, O_L2, O_R1 and the *lac* repressor sequence is shown to the left of the O_L2 helix. The corresponding sequence in O_R1 is shown to the left of the O_R1 helix. The two base pairs hidden by the backbone on each turn are shown to the right of the respective helices. The brackets indicate the G–C,A–T couplet feature in each helical sequence. The dashed A–T at the top of the O_R2 sequence is the same pair shown at the bottom of the O_R1 sequence; the top pair in the O_R3 sequence is the next pair following the pair shown at the bottom of the O_R2 sequence. The sequences shown for O_R1 and O_R3 are on the same face of the helix, when viewed as a continuum; whereas the sequence shown for O_R2 is on a face rotated 108° relative to that shown for O_R1 and O_R3.

to contain a repressor binding site, the 'leader region' of the tryptophan operon[12]. As noted above, the attenuator region is rich in G–C,A–T couplets; there are four additional couplets preceding the attenuator region, the first appearing 13 bases from the starting point of transcription. But only the second G–C,A–T couplet in the attenuator region is preceded by a sequence similar to that seen in the *lac* and λ repressor-binding regions (Fig. 3). The recurring face sequence seen in these five cases is

$$\begin{array}{l} \quad\binom{T}{A}\quad\ \binom{A}{T}\quad\ \binom{G}{G} \\ \mathrm{AA--CTC--GG} \\ \mathrm{TT--GAG--GC} \end{array}$$

The 5′–3′ orientation of the sequence is the same in *trp* and *lac* with the reverse orientation seen in the three λ sites.

The chance occurrence of the juxtaposition of a G–C,A–T couplet with a very similar sequence in all these cases is very low. Neither feature nor the combination of the two, however, provides the symmetry one would expect for recognition by dimeric or tetrameric repressor molecules. The 17-base-pair groups with strong rotational symmetry, observed in the analysis of Maniatis *et al.*[6], almost certainly serve this function. These are shown, in helical form, in Fig. 4. The faces shown here are rotated 36° relative to those shown in Figs 1 and 2 for O_L1, O_L2, and O_R1, 72° for O_R2 and O_R3. Thus the sequence similarities discussed above are seen on the face of the helix which is adjacent to the face in which a rotationally symmetrical group is seen in O_L1, O_L2 and O_R1. The symmetry groups themselves do not fall on a single face of the helix in either the rightward or leftward λ sites, for example, the face shown in

Fig. 3 Partial sequences from the *trp* attenuator region and the repressor binding sites of *lac*, O_L1, O_L2 and O_R1 have been aligned by the position of their G–C,A–T couplets. The base pairs in bold letters represent the "common" sequence; those pairs in light letters are not part of this sequence but are part of the G–C,A–T couplets.

TRP	LAC	O_L1	O_L2	O_R1
A-T	**T-A**			
T-A	**T-A**			
G-C	**G-C**			
A-T	**T-A**			
G-C	**G-C**			
G-C	**G-C**	G-C	G-C	G-C
G-C	**C-G**	**C-G**	**C-G**	**C-G**
G-C	C-G	**G-C**	**G-C**	**G-C**
		G-C	**G-C**	**G-C**
		T-A	**A-T**	**A-T**
		G-C	**G-C**	**G-C**
A-T	A-T	**T-A**	**T-A**	**T-A**
A-T	A-T	**A-T**	**T-A**	**T-A**
A-T	A-T	A-T	A-T	T-A

Fig. 4 for O_L2 lies at 144° relative to that shown for O_L1.

If the repressor-binding function is conceded to the rotationally symmetrical groups, what function can be suggested for the two recurrent features observed in this analysis? Furthermore, what mechanism would give rise to a sequence, shared, but with reversed 5′–3′ orientation in different cases? A case can be made that the G–C,A–T couplet may be part of a recognition sequence for RNA polymerase. From its appearances in known control sequences, it seems that it may be involved in both promoter and terminator sequences. Though the probability of a G–C,A–T couplet occurring somewhere in a given promoter sequence is high (probability of one or more couplets for a sequence of size *n* is $1-(31/32)^{n-13}$ or 0.42 for an *n* of 30), the probability of finding it in 11 such sequences, often several times over, drops well below 1 in 100,000. It is true that this feature does not always show the same position relative to the starting point of transcription, but the relationship of the initial binding point to the starting point is unclear at this time[6,13,14]. With regard to the terminator regions, the attenuator-terminated *trp* mRNA ends precisely with the last of the G–C,A–T couplets in the attenuator region[12]. A possible terminator region for the *tyr* tRNA sequence reads CCATC – – TTT – – – – – – – CCC on the 5′ → 3′ strand[15]. Here, as in O_L1 and O_R1, ATC appears in the groove preceding a G–C,A–T couplet but the order of the A–T triplet and the G–C triplet is reversed.

How does the "common" sequence fit in? This sequence is juxtaposed with the G–C,A–T couplet only in repressor-binding regions (the claim of the *trp* attenuator region here is tenuous, however) and not in the promoters. The homologous sequence observed by Pribnow[16] in six promoters appears in position 7 to 13 of O_L1 in Fig. 1, not in the common region. It is difficult to see why there should be this sequence appearing with the same 5′ → 3′ orientation in *trp* and *lac* and with the opposite orientation in O_L1, O_L2, and O_R1. Another puzzling observation, the reflection symmetry seen on the O_L1 face in Fig. 1, may hint at an explanation. This symmetry, though definitely non-random (random odds ~7/100,000), would seem of little use to a repressor dimer. If the reflection symmetry of O_L1 were perfect, however, one would have a sequence containing the "common" sequence of the *lac* and *trp* orientation in the upper half and the "common" sequence of the O_L1, O_L2, and O_R1 orientation in the lower half and a G–C,A–T couplet in each direction. Divergence from a common precursor sequence of this type could produce the shared features observed here and might be related to those homologies observed by Pribnow as well. If this evolutionary hypothesis is correct and similarly obtains in eukaryotes, it could provide the molecular basis of an integrator gene[16].

Consider the implications of this hypothesis for the *lac* and λ sequences. In *lac* it looks as though the common sequence is the recognition sequence for repressor; here the sequence has been duplicated in the lower half of the site (in place of the original reflected sequence?); in the λ sites it still forms parts of the basis of the recognition site but with a shift in reading frame to an adjacent face of the helix, preventing heterologous interactions. This view suggests that homologies would exist, as well, in the "dissimilar" halves of *lac* and O_L1. If the upper half of the O_L1 sequence is compared with the lower half of the *lac* sequence after the latter has been rotated 180°, one observes the rather remarkable pattern that there is a perfect match at every other position in the two sequences, that is, *lac* position 23 = O_L1 position 8, *lac* position 21 = O_L1 position 10, and so on.

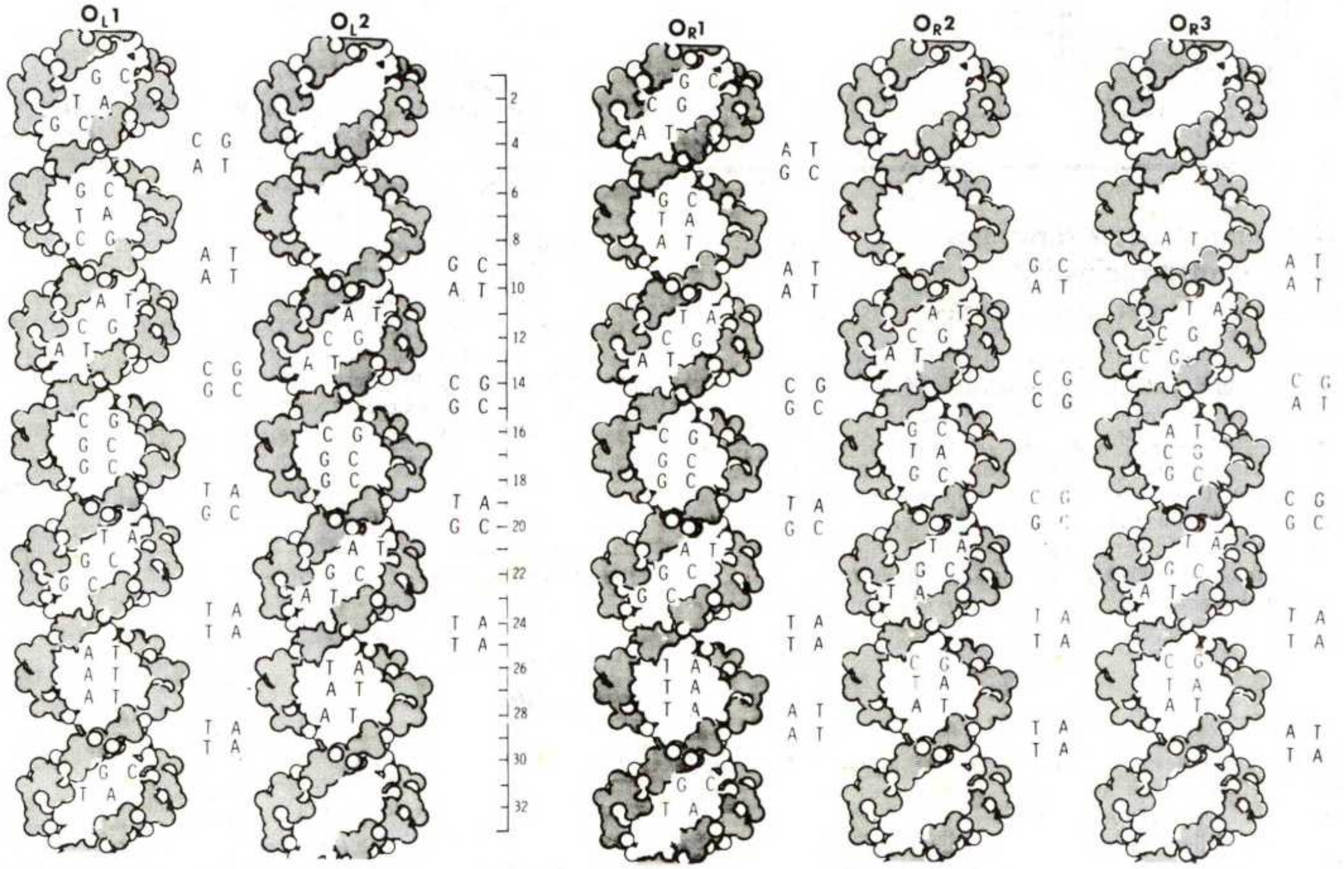

Fig. 4 In this view of the repressor-binding sequences of O_L1, O_L2, O_R1, O_R2 and O_R3, the rotational axis observed by Maniatis *et al.* has been located in the centre of the central wide groove in each case. The face shown here for O_L2 lies at a viewing angle of 144° relative to the face shown for O_L1. The face shown for O_R2 lies at a viewing angle of 144° relative to the face shown for O_R1; that for O_R3 lies at 252° relative to O_R1, 108° relative to O_R2. O_L3 (not shown) lies on the same face as O_L2. The sequences shown are contiguous; for example, the first base pair of O_L2 follows the last shown for O_L1. The rotationally symmetric 17-base-pair groups of Maniatis *et al.* lie in positions 9 to 25. The O_L1, O_L2 and O_R1 sequences have been moved upwards one position relative to Figs 1 and 2, whereas O_R2 and O_R3 have been shifted downwards two positions.

In conclusion, I suggest that synthetic DNA probes of the sequence

$$\begin{array}{lcccccc}
 & (\mathrm{C}) & (\mathrm{C}) & (\mathrm{C}) & (\mathrm{T}) & (\mathrm{T}) & (\mathrm{T}) \\
\mathrm{C\,G\,G\,T\,C\,A\,C\,C\,A\,T} & \mathrm{G} & \mathrm{G} & \mathrm{G} & \mathrm{A} & \mathrm{A} & \mathrm{A} \\
\qquad\qquad\text{and} & & & \text{- - -} & \text{- - -} & & \\
\mathrm{G\,C\,C\,A\,G\,T\,G\,G\,T\,A} & \mathrm{C} & \mathrm{C} & \mathrm{C} & \mathrm{T} & \mathrm{T} & \mathrm{T} \\
 & (\mathrm{G}) & (\mathrm{G}) & (\mathrm{G}) & (\mathrm{A}) & (\mathrm{A}) & (\mathrm{A})
\end{array}$$

in both 5′, 3′ permutations may prove very useful, in addition to that proposed by Maniatis *et al.*[6], in the investigation of promoters, terminators and repressor-binding sites.

This work was supported by a grant from the American Cancer Society (Maryland Division).

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Received August 4, 1975; accepted February 17, 1976.

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Detection of slow rotational motions of proteins by steady-state phosphorescence anisotropy

WE have used the anisotropy of the phosphorescence from intrinsic chromophores of proteins to detect slow rotational motions of the macromolecules in highly viscous solutions. This method has potential for monitoring the mobility of proteins in structures such as membranes and viruses.

Rotational motions of the emitting molecules in a solution result in the depolarisation or loss in anisotropy of the emission. The quantitative relationship between the emission anisotropy observed in conditions of constant illumination and the rotational time is embodied in the Perrin equation[1]

$$\frac{1}{A} = \frac{1}{A_0}\left(1+\frac{\tau}{\theta}\right) \tag{1}$$

When the rotational correlation time θ is equal to the excited state lifetime τ, the anisotropy A decreases to half A_0 the anisotropy for a stationary emitter.

Weber applied emission polarisation[2] to the study of protein hydrodynamics using fluorescent labelled protein molecules. Fluorescence polarisation is widely used in probing the rotational mobility of proteins and the flexibility of their active sites in steady-state experiments[3] and in nanosecond techniques[4-6]. Introduction of a fluorescent label is usually required in that the lifetimes of the intrinsic fluorescence from tryptophan and tyrosine residues are much shorter than the rotational correlation times of most proteins. Rotational correlation times as long as 500 ns have been measured by the use of fluorescence probes with 100–200-ns excited singlet lifetimes[7]. Rotational motions which are much slower than the fluorescence lifetime, however, are difficult to detect.

For access to slower rotational motions a long lived emission is necessary. Whereas long lived phosphorescence is usually quenched in fluid solutions, we have shown that buried tryptophan residues in proteins which are protected from quenching by solvent-mediated processes, phosphoresce in fluid solutions with lifetimes in the millisecond range[8]. As a consequence, rotational motions occurring on this time scale and shorter become amenable to experimental detection. We report here a preliminary study on the use of steady-state phosphorescence anisotropy measurements to monitor rotational motions of proteins.

The tryptophan residues in proteins were excited at 300 nm from a mercury lamp. Phosphorescence was measured on the 0–0 vibronic transition of tryptophan ($\lambda = 410$–415 nm) which has the largest emission anisotropy[9,10]. Measurements were obtained with a phosphoroscope described elsewhere[11], in which both the exciting and emission beams were polarised with Polaroid HNPs linear polarisers. Anisotropy A was calculated from the relationship

$$A = \frac{I_{11} - GI_1}{I_{11} + 2GI_1} \tag{2}$$

where I_{11} and I_1 are the emission intensities polarised parallel and perpendicular respectively to the vertically polarised exciting beam, and G is an instrumental correction factor[12]. The values of A_0 reported are the largest values of A obtained at temperatures where the glass had softened somewhat eliminating strains and cracks, but molecular rotational motions were still insignificant.

The anisotropy of the tryptophan phosphorescence for several proteins and for indole in glycol–H_2O were determined. The values for the anistropy of the 0–0 transition at temperatures below 120 K appear in Table 1. At these temperatures, near the glass transition temperatures of the solvents, molecular rotational times are longer than the 5–6-s tryptophan-triplet lifetime. These values of A_0 therefore, depend only on the electronic properties of the isolated chromophores and the extent of electronic coupling, or energy transfer, between chromophores. The connection between these limiting anisotropies and energy transfer between tryptophan residues in proteins in relation to earlier studies[9], and to observations of heterogeneity in protein phosphorescence spectra[13], will be considered elsewhere.

The rotational correlation time for spherical particles was

Table 1 Limiting phosphorescence anisotropy values A_0 and $T_{\frac{1}{2}}$-values of some proteins in 1:1 propylene glycol–buffer

	A_0	P_0	Molecular weight	$T_{\frac{1}{2}}$(°C)
Indole	−0.140	−0.22	117	−116
α-Chymotrypsin	−0.032	−0.05	23,000	—
DNase I	−0.095	−0.15	31,000	−96.5
BCA	−0.090	−0.14	31,000	−96.5
HSA	−0.140	−0.22	65,000	—
BSA	−0.140	−0.22	65,000	−93
LADH	−0.140	−0.22	80,000	−90
GBM	−0.102	−0.16		
S-13 viruses	−0.090	−0.14		
Erythrocyte ghosts	−0.083	−0.13		

P_0, the limiting degree of polarisation, is included for comparison with earlier works where the old notation was used. The relationship between anisotropy A and the degree of polarisation P, used in the older notation, is given by

$$\frac{1}{A} = \frac{3}{2}\left(\frac{1}{P} - \frac{1}{3}\right)$$

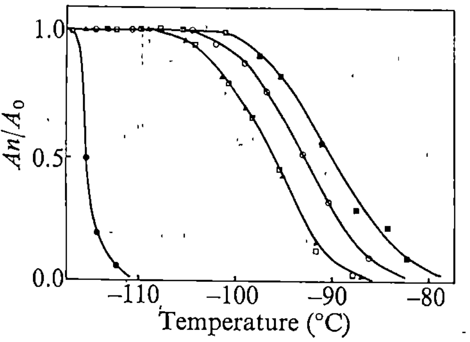
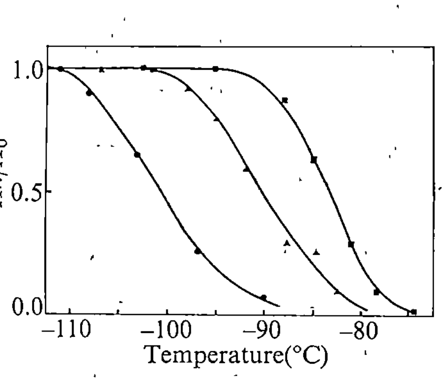


Fig. 1 Relative anisotropy of tryptophan phosphorescence as a function of temperature for globular proteins in 1:1 propylene glycol–phosphate buffer (0.05 M, pH 7). □, DNase I (Worthington); ▲, bovine carbonic anhydrase (Sigma); ○, bovine serum albumin (Worthington), ■, alcohol dehydrogenase from horse liver (Worthington). The anisotropy curve of indole (●) in the same solvent is included for reference All were at 10^{-4} M. In independent experiments *An* varied by less than 10%.

shown by Einstein[14] to be equal to $v\eta/kT$ where v is the volume of the particle, η is the viscosity and k and T are the Boltzman factor and temperature (K) respectively. In polar solvents at low temperatures there is a steep dependence of viscosity on temperature, so that the temperature dependence of the rotational correlation time reflects principally changes in viscosity. As the viscosity of the glycol–H_2O solvent decreases at higher temperatures, rotational motions of proteins manifest themselves with a decrease in the magnitude of A. Equation (1) shows that A becomes a measure of the relative mobility of various proteins if the lifetime remains constant. The triplet lifetimes, however, decrease at warmer temperatures and vary from protein to protein. Each of the anisotropy values, therefore, was corrected to the value An it would have at a fixed lifetime, arbitrarily taken as 3 s.

Figure 1 shows the variation of An with temperature for indole and for several proteins. The decline in phosphoresence anisotropy is observed at relatively low temperatures, or high viscosities. The temperature $T_{\frac{1}{2}}$ at which An/A_0 is reduced to one half is compared with the molecular weight of the protein in Table 1. As the volume of globular proteins increases with molecular weight, there is a concomitant increase in the temperature at which depolarisation occurs. The trend shown by molecular volumes compared with $T_{\frac{1}{2}}$ indicates that it is rotational diffusion of the entire macromolecule, rather than independent motions of the aromatic side chains, which is the dominating factor in depolarising the intrinsic phosphorescence of proteins. That the temperature–anisotropy profiles depicted in Fig. 1 for various proteins reflect a viscosity-dependent process is confirmed in Fig. 2. As the solvent viscosity is increased from ethylene glycol–H_2O to propylene glycol–H_2O to glycerol–H_2O, there is a corresponding increase in the temperature range for which depolarisation occurs with a given protein. In addition, experiments (to be published) in which phosphorescence depolarisation is followed as a function of time at a given temperature, unambiguously establish rotational motions as the origin of these transitions.

Fig. 2 Relative anisotropy of tryptophan phosphorescence as a function of temperature for alcohol dehydrogenase from horse liver in solvent mixtures of different viscosity. ●, Ethylene glycol–phosphate buffer (1:1); ▲, propylene glycol–phosphate buffer (1:1); ■, glycerol–phosphate buffer (2:1)



When the present steady-state method of measuring phosphorescence anisotropies is used, the rotations of even relatively large proteins must be slowed down by means of high viscosities to observe the depolarisation. The technique suggests itself as a method for measuring rotational motions of larger macromolecular complexes or of proteins embedded in media of high viscosity where the relaxation times can be expected to be long. Figure 3 illustrates the feasibility of using phosphorescence anisotropy to monitor the rotational mobility of membrane and viral coat proteins. The emission anisotropy for proteins in rat glomerular basement membrane (GBM), human erythrocyte ghosts and the coat of S-13 bacteriophage are plotted as a function of temperature along with the data for indole.

In contrast with the behaviour of the soluble proteins and the glycoprotein in GBM, the tryptophan phosphorescence of erythrocyte ghosts depolarises in stages rather than in a single transition. With increasing temperature an initial loss of anisotropy occurs at a viscosity at which soluble proteins (molecular weight 60,000–80,000) undergo depolarisation. The remaining anisotropy of the phosphorescence from the ghosts decreases slowly with increasing temperature. Only at much lower viscosity, about 0 °C, is most of the anisotropy lost. The data suggest that some proteins are highly constrained through interactions between macromolecular components although others are relatively free to undergo independent rotational motions, and probably correspond to specific types of membrane proteins at the surface. Selective removal of particular proteins[15] followed by phosphorescence measurements would establish the mobility of various membrane

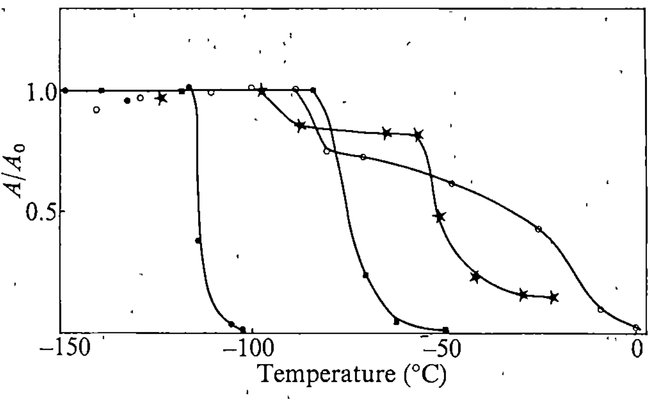


Fig. 3 Relative anisotropy of tryptophan phosphorescence as a function of temperature for biological structures suspended in propylene glycol-phosphate buffer (1:1). ■, GBM from rat kidney. They were prepared as previously described[23], in the laboratory of Dr N Kalant at the Lady Davis Institute for Medical Research. ★, S-13 bacteriophage viruses, a gift of Dr J. H. Spencer, Department of Biochemistry, McGill University. The method for growth, collection and purification has been described before[24]. ○, Human erythrocyte ghosts were given by Dr Kahlenberg and were prepared by the method of Dodge *et al.*[25]. With these suspensions the O_2 concentration of the solvent was reduced by bubbling the samples with highly purified N_2 (Union Carbide, Canada, 99.997% N_2) after which the 5-mm outer diameter, round, Spectrosil quartz sample cells were sealed. ●, Indole anisotropy curve in the same solvent is included for reference.

proteins and contribute to the elucidation of their role in membrane structure.

There is a similar discontinuous temperature profile of the phosphorescence anisotropy of the coat proteins of S-13 bacteriophage. The data establish that in the sample investigated, several rotational correlation times are involved. While the results may reflect some independent motion of the individual coat proteins in the virus capsule, due to the rather harsh deoxygenation treatment of this sample (bubbling with N_2) the observed heterogeneity probably arises from damaged and aggregated phage particles. The depolarisation profile is included to reflect the ability of the technique to detect heterogeneous rotational correlation times and to follow the motion of large macromolecular complexes. The anisotropy–temperature profile for GBM from rat kidney reveals that tryptophans are not rigidly fixed within the structure in that loss of anisotropy is observed at a temperature characteristic of soluble proteins (molecular weight ~ 100,000). GBM is composed primarily of collagen and one or more glycoproteins[16]. The glycoproteins therefore cannot be rigidly fixed within the insoluble basement membrane matrix but must have considerable rotational mobility of their own.

Several features of this technique for monitoring rotational motions of proteins are noteworthy. (1) Rotational motions are detected by the use of the depolarisation of the emission from intrinsic chromophores in proteins, thus avoiding labelling of proteins with probes. There are no concerns about the extent of perturbation to the system and the possibility of independent motions of the labelling group itself. A perturbation has been introduced instead with the use of glycol–water solvents. Studies of the biological activity[17,18] and the X-ray analysis[19,20] of proteins in these solvents, however, suggest that the perturbation is small.

(2) In the investigation of more complex systems involving two or more proteins, such as with the erythrocyte ghost membranes, the use of the intrinsic emission offers the possibility of resolving the rotational correlation time into contributions from individual proteins. Selective observation of particular proteins is also possible. With structures such as GBM the tryptophan phosphorescence arises only from the glycoprotein component[21] in that tryptophan is generally absent from collagen[22].

(3) The method is useful for investigating slow rotational motions. The functioning of proteins not only as soluble cellular entities but within macromolecular complexes and in insoluble structures, such as membranes and muscle fibres, makes necessary the development of techniques to elucidate the structure and dynamics of such systems. We feel that phosphorescence anisotropy measurements can contribute to the understanding of the molecular basis of the function of such systems.

This work was supported by a grant from the National Research Council of Canada.

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Received September 15, 1975; accepted February 6, 1976

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Errata

In the article "Eukaryotic DNA replication complex" by V. M. Genta, D. G. Kaufman, W. K. Kaufman and B. I. Gerwin (*Nature*, **259**, 502; 1976) in the last paragraph, lines 8–13 should read . . . the observation of ATP-dependent DNA synthesis in M-band fractions isolated from sea urchin embryo nuclei[14]. Since nuclear membrane is largely confined to the LB (unpublished observation), the result reported here differs from studies involving the M-band technique[14–16] because . . .

In the article "Dissociation of response to injected gonadotropin between the Graafian follicle and oocyte in pigs" by R. H. F. Hunter, B. Cook and T. G. Baker (*Nature*, **260**, 157; 1976) the first entry in the 7th column of Table 1 should read 81.2% and not as printed.

In the article "Gibberillic acid enhances the level of translatable mRNA for α-amylase in barley aleurone layers" by T. J. V. Higgins, J A. Zwar and J. V. Jacobsen (*Nature*, **260**, 166; 1976) Figs 1 and 3 have been transposed. The legends are correct as they stand.

In the article "New H_2O celestial sources associated with H II regions in the Southern Hemisphere" by P. Kaufmann *et al.* (*Nature*, **260**, 306; 1976) the second entry in the 1st column of Table 1 should read 330.9–0.4 and not as printed.

In the article "Effect of surface roughness on rolling friction and adhesion between elastic solids" by G. A. D. Briggs and B. J. Briscoe (*Nature*, **260**, 313; 1976) the first line under the equation in the first paragraph should read : where σ is the distribution of asperity heights . . .

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matters arising

Polymerisation and the solar neutrino problem

JACOBS[1] has questioned the efficiency of the helium flushing method for extracting Ar^+ ions produced in the reaction

$$\nu + {}^{37}Cl \rightarrow e^- + {}^{37}Ar^+$$

He suggests that Ar^+ ions could induce polymerisation in the liquid C_2Cl_4 and get trapped before they are neutralised by capturing electrons, and consequently all the Ar^+ ions produced in the above reaction are not detected.

Tetrachloroethylene is an unsaturated molecule which undergoes polymerisation, when irradiated by MeV γ rays, at the rate of $\sim 2.1 \times 10^{-10}$ mol ml^{-1} s^{-1} (based on the dose rate of 1.5×10 eV ml^{-1} s^{-1} and the polymer yield in the radiation experiments)[2]. It is known that halogenated ethylenes undergo predominantly free-radical polymerisation, the cationic initiation of the chain is less likely because of the presence of electronegative groups in C_2Cl_4 leaving an electropositive carbon atom at the polymerisation site. Even if Ar^+ can initiate polymerisation, the rate will be much smaller than that quoted above for γ rays. On the other hand, since the electron affinity of Ar^+ is high, the Ar^+ radical produced by the solar neutrino can be neutralised at a very fast rate. The electron transfer between the neutral radical and the positively charged ion is diffusion controlled with the rate constant 10^9–10^{10} l mol^{-1} s^{-1}, while the rate constants of generalised polymerisation[3] are of the order of 1–30 l mol^{-1} s^{-1}. It is clear there will be negligible trapping of Ar^+ ions through polymerisation in the tank.

The foregoing arguments receive indirect but unambiguous support from two experiments: (1) Barabanov *et al.*[4] have measured the relative efficiency of removing a small number of ^{37}Ar atoms by helium purging in liquid C_2Cl_4 and in solid C_6Cl_6 by irradiating both the substances with fast neutrons from a PuBe source. They find that for the same number of fast neutrons, the same number of ^{37}Ar atoms is produced. Considering the different physical and chemical properties of C_2Cl_4 and C_6Cl_6, the probability of identical rate of trapping is surely negligible. (2) In the helium-purging efficiency experiment of Davis[5], liquid C_2Cl_4 was irradiated with neutrons from an RaBe source to get an apparent ^{37}Ar production rate per incident neutron of 7.5×10^{-7}. If chemical trapping is important, the true production rate should be at least an order of magnitude higher than the observed one. The cross sections for the nuclear reactions

$$n + {}^{35}Cl \rightarrow {}^{35}S + p \text{ (fast)}$$
$$\hookrightarrow p + {}^{37}Cl \rightarrow {}^{37}Ar + n$$

are sufficiently well known to render a theoretical calculation of the true production rate of ^{37}Ar possible; we find it to be $\sim 4 \times 10^{-7}$, in reasonable agreement with Davis' measurements.

We may add to these the very careful analyses by Wolfendale *et al.*[6] and by Cassiday[7] of the cosmic ray-induced background in Davis' experiment. These analyses lead to values for the photonuclear cross section which are in substantial accord with other cosmic ray and accelerator measurements. If the normalisation of the background at the depth of 25 m.w.e. (metres water equivalent) is wrong as implied by Jacobs, this would hardly have been the case. The conclusion thus seems inescapable, that chemical trapping of any kind—polymerisation or other chemical process—is of negligible significance in Davis' experiment.

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JACOBS REPLIES—In a brief communication[1] I explored the logic of the possibility that the solar neutrino problem might be caused by the chemistry of the solar neutrino experiment. I concluded that this chemical hypothesis was viable only if $^{37}Ar^+$ became 'chemically trapped' in Davis' system; five mechanisms for trapping the argon in C_2Cl_4 were suggested.

Banerjee *et al.*[2] criticise one of my trapping mechanisms—induced polymerisation in the liquid tetrachloroethylene—and imply by heuristic arguments that Davis' neutron-irradiation test[3] and the cosmic-ray background test[4,5] completely rule out the possibility of any significant 'chemical trapping'. I suggest that the conclusions of Banerjee *et al.* are comfortably plausible, but they are by no means logically inescapable.

Induced polymerisation of C_2Cl_4 is the least tenable of my suggested trapping mechanisms; the large atomic radius of chlorine will hinder the formation of any long chain polymer[1]. It is much more probable that the argon is 'chemically trapped' in a small bound molecule or ion–molecule. One could just as easily interpret the results of Barabanov *et al.*[6] as positive support for the formation of $ArCl_n$, with $n = 2$, 4 or 6.

Order-of-magnitude arguments are unsafe in this controversy. The uncertainties intrinsic to present-day solar models can easily render acceptable a theoretical solar neutrino rate as low as 4 SNU (ref. 7), whereas Davis' latest two runs (36 and 37) indicate an experimental rate of $\sim$4 SNU—with a 1σ upper limit of $\sim$1.5 SNU for the average of runs 21–37 (ref. 7; estimated by eye from their Fig. 2). Thus, 'chemical trapping' need not hide 9 of every 10 argon atoms produced by solar neutrinos, but could well conceal only 60% of the argon on average.

Therefore, order-of-magnitude (or even crude qualitative) agreement between theory and experiment in some of the crucial tests[3-5] is not sufficient to eliminate the chemical hypothesis—only precise quantitative matching can do that. Finally, I conclude again that my chemical solution to the solar neutrino problem remains a viable alternative.

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Sub-microscopy porosity and superfluid ^4He

VAN Brakel and Heertjes[1] have reported on transfer phenomena in porous media, using the capillary rise of a wetting liquid. I would like to add to this by mentioning a phenomenon apparently similar to capillarity, although fundamentally different: the behaviour of superfluid ^{4}He in narrow gaps. This might contribute to the knowledge of transfer phenomena.

The behaviour of superfluids is not as well known as capillarity of a normal viscous wetting liquid. The viscosity of superfluid helium is zero. This provides a method[2] to detect and to estimate the size of sub-microscopic pores in solid materials as well as in small, packed grains. Those materials with small enough pores and through which only the superfluid helium flows are called superleaks.

When liquid ^{4}He is cooled to a temperature of 2.17 K, the λ point, there is a phase transition, and some of the liquid becomes superfluid. As the temperature decreases further, the proportion of superfluid increases. The temperature at which the superfluid starts to flow through a superleak is called the onset temperature and is always below the transition temperature. The difference between the two temperatures is the 'depression of the transition temperature'. The smaller the pores the lower the onset temperatures.

The channels through a superleak are not uniform but are at random, interconnecting each other. If φ_m is the minimum diameter along a connection path, then the largest φ_m of all possible paths determines the onset temperature, because in that diameter superfluid movement will occur first, that is, at the highest temperature below the λ transition. This is a 'size effect' and arises from interactions between excitations of quasi particles of the liquid helium and the solid. When the temperature is high and the gap or diameter is small, the interaction is equivalent to the presence of viscosity. The number of excitations diminishes with the temperature.

To estimate the widths of the gaps, or the 'diameters' of the pores through which the superfluid penetrates the superleaks, the following two relationships between the depression ΔT_λ of the transition temperature and the width d of those gaps are used

$$\Delta T_\lambda \simeq -\frac{2\times10^{-14}}{d^2}\ \mathrm{K}$$

$$\text{or } \Delta T_\lambda \simeq -\frac{2.5\times10^{-11}}{d^{3/2}}\ \mathrm{K}$$

if d is expressed in cm. The first relationship was derived by Ginzburg and Pitaevskii[3] and the second, which seems to be more consistent with the experimental data, by Mamaladze[4]. It was, however, pointed out[5] that the width of a parallel-sided channel, which probably occurs most frequently, is $2d$. Using this latter criterion and the two expressions given above, the width of the channels in a granite and a limestone, which were found to be superleaks, was estimated for both between 34 Å and 22 Å (ref. 6) and the onset temperatures measured were 1.48 K and 1.45 K, respectively.

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Antibiotic resistance in laboratory workers

RECENT publications by Williams Smith[1] and Anderson[2] have been concerned with the ability of *Escherichia coli* K12 to survive in the human alimentary tract. This is a matter of importance since a detailed knowledge of the behaviour of this much used *E. coli* strain is needed to assess the potential hazard of many genetic engineering experiments.

In this department we have monitored the faecal flora of laboratory workers engaged in work with R-plasmids to see whether they have ever excreted the K12 strains with which they were working, or other *E. coli* carrying plasmids which were being studied in this laboratory. Most of our experiments have involved a restricted number of R-plasmids (Table 1) in *E. coli* UB1139, a nalidixic acid-resistant derivative of *E. coli* K12.

A total of 52 samples was taken from the two individuals under study during a period of 184 d. Suspensions of faecal material in physiological saline were plated on McConkey agar to determine the total coliform count, and on agar containing either ampicillin (25 μg ml^{-1}), streptomycin (10 μg ml^{-1}), tetracycline (25 μg ml^{-1}), chloramphenicol (25 μg ml^{-1}) or nalidixic acid (30 μg ml^{-1}). Resistant colonies arising on these plates (if any) were then tested for indole production in peptone water, and for acid+gas production in McConkey broth after overnight incubation at 44 °C (refs 3 and 4). If available, ten resistant colonies from each plate were O-antigen typed[5] and tested for the transferability of their resistance patterns by mating with a standard *E. coli* R$^-$ (ref. 5). In all, 590 isolates were examined in this way.

A total of 38 R-plasmid carrying *E. coli* have been isolated so far, and seven different resistance patterns have been encountered (Table 1). None of these resistant *E. coli* was UB1139, nor were the resistance patterns encountered those of the plasmids used for experimental purposes.

The workers concerned in these studies used conventional procedures for testing low-risk bacteria[6,7]. Mouth pipetting was not used but experiments were conducted in the open laboratory. These results therefore reinforce the views of Williams-Smith[1] and Anderson[2] that *E. coli* K12 lines are unlikely to establish themselves as major components of the faecal flora of workers handling these bacteria so long as reasonable laboratory procedures are used.

Table 1 Comparison of the resistance patterns of the plasmids isolated from faecal samples with the patterns of the experimental plasmids used by the workers concerned

Faecal samples		Experimental plasmids	
Pattern	No. of times isolated	Plasmid	Pattern
Tc	23	R1*drd*19.K1	Kn
Sm Su	3	R46	Tc Sm Sp Ap Su
Tc Su	1	R55-1	Su Cm
Sm Ap Su	1	R64-11	Tc Sm
Tc Sm Su	8	R100-1	Tc Sm Su Cm
Tc Sm Ap Su	1	R388	Su Tp
Tc Sm Ap Su Cm	1	R391	Kn
		R751	Tp

Total number of isolates examined: 590. Abbreviations for resistance markers: Tc, tetracycline; Sm, streptomycin; Ap, ampicillin; Su, sulphonamide; Cm, chloramphenicol; Sp, spectinomycin; Kn, kanamycin; Tp, trimethoprim.

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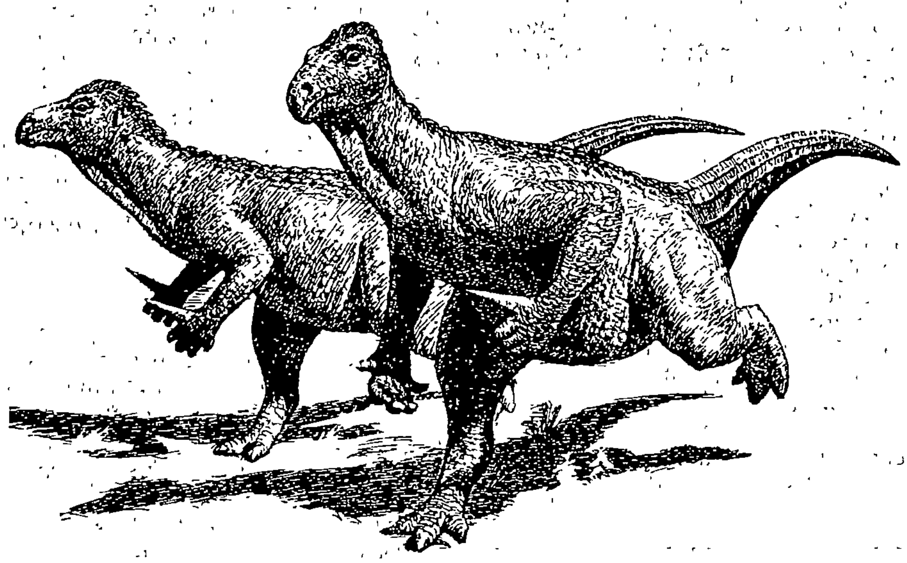
Dinosaur teleology

L. B. Halstead

FOR 140 million years the dinosaurs dominated the continents, then 64 million years ago they suddenly disappeared. These animals have always been accepted as the acme of the world of cold-blooded reptiles. In recent years, however, R. T. Bakker has been arguing that their former success was a consequence of their high metabolism and fast energy production (*Nature*, **238**, 81–85, 1972). He considered that they were warm-blooded in the same sense as birds and mammals, maintaining a constant internal temperature (homoiothermy) by means of their own internal activity (endothermy) Adrian Desmond's *Hot-Blooded Dinosaurs** is a fascinating account of the history of the study of dinosaurs and the changing views of their physiology, culminating in a vigorous exposition of Bakker's ideas. As an exercise in evangelism it is exemplary.



Desmond uses the old legal trick of the leading question How could *Tyrannosaurus* have engaged in lengthy gladiatorial combats with *Triceratops*, if they had not had a high metabolic rate? How could *Struthiomimus*—the ostrich dinosaur—have maintained a speed of 80 km h^{-1} with the physiology and energy output of a lizard? The answers quite clearly are that they could not; but what Desmond fails to establish is that such behaviour actually took place or was even necessary

All the evidence marshalled to support the notion of dinosaur endothermy is capable of alternative interpretations. The stance and gait of dinosaurs with the limbs positioned beneath the body is comparable to that found in birds and mammals This stance seems to be associated with endothermy today. There is therefore a *prima facie* case that the energy requirements may have been the same; yet the crocodile's high walk is not evidence for endothermy—there is an advantage in an upright posture *per se*.

Much emphasis has been made of the different predator–prey ratios between living ectothermic and endothermic populations. If one examines mammalian ratios of kg predator/kg prey, it can be seen that this changes from small animals to larger. The 'endothermic' situation with the dinosaurs could be interpreted as a direct consequence of their large size.

Perhaps the most striking similarity between dinosaurs and mammals is found in the microstructure of their bone. The laminar bone of sauropod dinosaurs is indistinguishable from that of some of the larger artiodactyls and it is presumed that this indicated a similar metabolism. Such microarchitecture may in fact be simply due to the rate of bone formation and is thus characteristic merely of large animals. The large dicynodonts with their sprawling gait (and therefore not comparable in terms of their locomotion to the dinosaurs) have the identical bone histology The evidence for endothermy from bone histology is not particularly convincing

The existence of dinosaurs in latitudes that were situated in the Cretaceous Arctic would seem at first glance to be strong evidence for endothermy. To speak of the Arctic at the time of the dinosaurs is a little misleading as there were no polar ice caps The temperature of the Cretaceous seas at differing latitudes has been ascertained from oxygen isotope studies and the mean sea level air temperatures subsequently calculated The range from Equator to Arctic was 31–14 °C. It is not unreasonable to imagine the odd dinosaur surviving such conditions

The final piece of argument concerns the first bird *Archaeopteryx* and the pterosaurs. Both types of animal originated from the same basic stock and the birds seem to be directly descended from the dinosaurs Birds and pterosaurs had an insulating covering as did the advanced paramammals and primitive mammals In all these groups, it is accepted that they possessed a high metabolic rate and were genuine endotherms. Desmond contends that as *Archaeopteryx* was skeletally similar to small theropod dinosaurs and possessed feathers primarily for insulation, then perhaps such a covering was a common feature of all these animals.

Once the premise that dinosaurs must have been endotherms is accepted, it is but a short step to remove them from the Reptilia and place them in a new class, the Dinosauria, which includes the birds Aves (Bakker and Galton, *Nature*, **248**, 168–172, 1974). Desmond for his part proposes the new superclass Endosauropsida to encompass the classes Dinosauria (including Aves) and Pterosauria.

The case for dinosaur endothermy has certainly not yet been convincingly established and, even if it were, there would still be no justification for such a drastic restructuring of the classification of the vertebrates Even the man in the street has some inkling of what a bird is and in no sense does this equate with dinosaurs Brontosaurs and

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**The Hot-Blooded Dinosaurs*: *A Revolution in Palaeontology* By Adrian J. Desmond Pp 238. (Blond and Briggs: London, November 1975.) £5.95.

Dr Halstead is a reader in geology and zoology at the University of Reading, UK.

blackbirds have precious little in common beyond sharing an ultimate common ancestor. The evolutionary line of the mammals can be traced back to the paramammals, but this is not a sound basis for terming the mammal-like reptiles 'mammals', nor for including the mammals in the Paramammalia. The fact that we can now begin to indicate lines of descent is not a licence to redraft the classification.

The conclusions drawn by Desmond run far ahead of the available evidence A great deal more work will be required before such notions of dinosaur endothermy can be taken seriously. There can be little doubt, however, that the dinosaurs were warm-blooded. The dinosaurs achieved a passive type of homoiothermy by being large; their surface–volume ratio would have ensured a fairly constant internal temperature. All the features that have been used to attribute endothermy to the dinosaurs can more easily be interpreted as being a direct consequence of their size.

It is a pity that Desmond's undoubted literary talents were not used in a more deserving cause than promoting hot-blooded dinosaurs. □

Synaptic receptors

Synaptic Receptors: Isolation and Molecular Biology. (Modern Pharmacology–Toxicology: A Series of Monographs and Textbooks.) By Eduardo de Robertis. Pp. xiv+387. (Dekker: New York, 1975.) $29.50.

In those synapses with chemical transmission, the transmitter substance acts on the postsynaptic membrane by combination with synaptic receptors. Within the last ten years, our knowledge of these receptors has advanced from a condition in which their existence as discrete structures was far from established to a situation in which several have been isolated. Their isolation is the subject of Dr de Robertis' book.

Synaptic receptors, as constituents of membranes, are amphipathic and there are, broadly speaking, two approaches to their isolation. The first uses detergents in aqueous solution to counteract their hydrophobicity and effect solubilisation. The second approach seeks to take advantage of this hydrophobicity by extracting receptors into nonaqueous solvents of low polarity. Dr de Robertis has played a dominant role in the development of the latter approach, whereas most other work has concentrated on the former. Recently, Barrantes *et al.* (*Nature*, **256**, 325–327, 1975) have shown that, at least in the case of the acetylcholine receptor, there is nothing in common between the material isolated by these two methods. This casts a long shadow over the book, for in spite of a superficial balance, it is, in fact, an apologia for Dr de Robertis This should surprise no one for Dr de Robertis' prolificity is truly remarkable, having isolated some half dozen receptors from twice as many tissues.

The need for an expensive monograph at the present time is open to the strongest doubt, more so since Dr de Robertis has displayed the virtue of brevity in a subsequent review covering the same ground (*Rev. Physiol. Biochem. Pharmac.*, **73**, 9–38; 1975). The book contains a quite incredible number of typographical errors from the merely irritating to the downright misleading.

Until such time as our knowledge of synaptic receptors enables distinction between the permanently valuable and the ephemeral, a book of this kind is prey to premature obsolescence and, at the price, just not worth it.

David Green

Conceptual shorthand

Transport Phenomena in Medicine and Biology. By M. Min-Shing Lih. Pp. xviii+531. (Wiley–Interscience: New York and London, 1975.) £14.70.

This book gives a very clear presentation of the mathematics necessary to describe—with a fair degree of rigour—the underlying physics involved in physiological processes concerned with transport of mass, momentum and energy. The author is to be complimented on achieving his declared aim of demonstrating how the mathematical conceptual 'shorthand' of physics can reduce the apparent phenomenological diversity of physiology. From the start the emphasis is on translation from the real world to the mathematical model, solution of the problem and back to the real world to present the answer in physical terms. The mathematics is continuously related to reality so that an experimentalist has his interest maintained and is carried along with the argument. This is done in a direct and refreshingly conversational way, producing more the flavour of a verbatim record of a colloquium than a concise university text.

With such a discursive style the author does well to contain his subject within 500 pages. The first five chapters present the mathematics necessary for a proper description of material processes in terms of continuum physics Chapters 6, 7 and 8 show us how to apply the mathematics to changing conditions as diverse as pulsatile blood flow, body heat loss, placenta mass transport and gastric acid analysis. Chapters 9 and 10 are specifically dedicated to haemorrheology—the non-Newtonian behaviour of blood as a suspension of cells flowing through narrow vessels—and oxygen transport This last chapter is an impressive discussion of a vital process, including examples of varied problem solving from membrane blood oxygenators to the oxygen–tension profile in the cornea.

We are told that the original manuscript was 'test-used' in a Chatauqua-type programme, and I would agree that the final book is very well suited for this self-help with guidance type of learning. The comprehensive presentation of 'Mathematical Background' in Chapter 2 makes the book self-contained. The reader is taken from functions and variables (p16) through to the tensor and its operations (p96), and by this time is ready to accept quite naturally the need for a tensor as a description of the stress on an element of fluid. I particularly liked the use of small print flow diagrams to illustrate on one page a single application of the mathematics being discussed. All of the mathematical development is integrated with the rest of the book through the use of problems.

Because of the excellent integration I wondered if it might not be difficult to easily 'dip' and use parts of the book as a companion text in an undergraduate course—say haemodynamics. For example, the pursuit of generality in the development of the mathematical models means that to arrive at a particular point such as the description of pulsatile flow in a pipe seemed to be longer than Womersley's direct approach. The very applied flavour, however, of the maths makes the attempt an attractive/worthwhile proposition. Certainly I have not found any more suitable text for that type of course.

Some of the material in this book will be familiar to any teacher in this field and even the organisation may in part be already in use. Yet the whole achievement of keeping the practical biomedical quality of the mathematics always so apparent makes this a most welcome book. It will certainly be a great help in courses at final special honours or MSc level in this country. Although the fly-leaf suggests it is an ideal text for the student I do not believe that it is an easy book for the unaided beginner. For the postgraduate student and the biomedical researcher it should handsomely repay the investment of time necessary to come to terms with this very able and challenging presentation.

R. G. Gosling

Chemical Fortran

Fortran IV in Chemistry: An Introduction to Computer-assisted Methods. By G. Beech. Pp. x+303 (Wiley: London and New York, October 1975.) £8.75.

UNDERGRADUATE chemistry courses have so far not shown as much response to the advent of the computer as engineering courses, and they do not reflect its importance as a research tool. It is still possible to teach both theory and laboratory techniques without reference to the computer; the main impetus towards Fortran programming and computer-assisted techniques lies in the knowledge that nearly every chemistry graduate soon becomes involved with computers. Dr Beech's book will be invaluable to those responsible for the introduction of these methods at undergraduate level, and essential reading to undergraduates and research students meeting them for the first time. Perhaps the most striking feature of the book is the care with which scopic data (including curve addition and reduction), crystal field simula- the chemistry and the underlying mathematics are developed as well as the computing. The operation of the programs listed is always fully described in the text.

An elementary knowledge of Fortran is assumed. The first chapter gives those elements not met on a first programming course, and those which may depend on the particular machine used. Next, normal numerical and statistical methods are given, including non-linear equations and a particularly clear account of matrix operations and eigenvalues. Practical examples of the application to experimental data are taken from chemical kinetics, electrochemistry, thermal analysis, multicomponent spectroscopic data and mass spectroscopy. The next chapter covers a very wide range of computer simulations of experimental observables: modelling simple kinetic and radiochemical systems (including Monte Carlo techniques), thermodynamics and equilibria, crystal lattice energies, statistical mechanics, spectrotions and nuclear magnetic resonance line splitting and intensity. Theoretical topics treated include particle-in-a-box (one dimensional with bond length alteration, and two dimensional) and atomic and molecular orbitals (LCAO and Huckel calculations). In this section, emphasis is rightly placed on plotting out the results.

Data acquisition by analogue to digital instruments is described, followed by data processing (including convolution smoothing and baseline corrections), illustrated by application to nuclear magnetic resonance spectra. Complex data systems are described for mass spectrometry, gas chromatography and γ-ray spectrometry. The book closes with a useful review of the literature, including handling chemical information on the computer. Each chapter is followed by problems and a bibliography. The book is well produced, and in view of its extensive coverage, good value. It is warmly recommended for both undergraduate and postgraduate use. **B. P. Levitt**

Changing role of ion beams

New Uses of Ion Accelerators. Edited by J. F. Ziegler. Pp. xii+482. (Plenum: New York and London, 1975.) $33.60.

THE current state of world economics demands that equipment, manpower and money are used as efficiently as possible. Nuclear physics has long been singled out as one of the 'big science' areas and more than a generation of expansion has provided a legacy of ion accelerators of which the low energy machines (that is about 5 MV and below) are used increasingly today in fields far removed from the compilation of nuclear structure data. But the story now is not merely one of keeping highly specialised machinery occupied. So interesting and valuable have the applications of ion beams to materials science and solid-state physics turned out to be that many Van de Graaff accelerators, for example, are now used exclusively in analytical experiments complementing (or replacing) more well established techniques. An analogous situation exists in the new field of ion implantation where exciting property changes are induced with ions usually below 500 keV in a variety of materials. The result is that nuclear physics laboratories are undergoing a metamorphosis towards solid-state applications which encompass physics, chemistry, materials science and metallurgy.

This book covers new developments in analytical methods, such as Rutherford backscattering, channelling, nuclear reactions and characteristic X rays for composition analysis, as well as ion implantation and ion-induced X rays. The editor deserves praise for welding the wide-ranging subject matter so satisfactorily; and it is perhaps significant that nearly four-fifths of the book relates to analytical techniques since this is the area in which ion accelerators are making immediate impact. The previous volume—which had a similar title—appeared eight years ago It was an excellent monograph which should have been more widely disseminated and its publication set the stage for constructive interaction between accelerator users and other physicists. It is encouraging to see how much ground has been covered in the intervening period and it is interesting to note the change of emphasis In the present volume, for example, nuclear astrophysics and beam foil spectroscopy give way to superconductivity, corrosion studies and pollution species detection.

The principal value of this book is the bringing together of the detailed procedures for backscattering and X-ray data collection and analysis, as well as up-to-date overviews of current developments in ion implantation and X-ray excitation theory. It is a rare thing to find accounts written from what is clearly first-hand experience of the systematics of establishing a first-class analytical service using an accelerated beam of protons or helium ions. Inevitably in a book consisting of contributed chapters there is duplication but the essentials are there, and numerous real-life examples are included. Surprisingly there is very little reference to the ion implantation of semiconductors. Perhaps it was felt that this important field (virtually synonymous now with advance in device technology) receives adequate cover elsewhere. As semiconductor implantation, however, has provided the bread and butter of the industrial side of ion implantation for many years, and is likely to continue doing so, an omission of this sort is regrettable. It is unfortunately made all the more obvious since the contributed chapter on superconductors is wrongly labelled 'semiconductors' in the text! Rutherford backscattering should be referred to as such to distinguish elastic Coulomb interactions from inelastic reactions arising within the nucleus (nuclear backscattering). Although they receive attention in separate chapters, the two are referred to a little indiscriminately elsewhere. Having said that, the treatment and power of such methods of analysis (and the two chapters on ion-induced X-ray physics) are excellently covered.

Accelerator users, present and intending, should reach for this volume No-one contemplating an integrated analytical programme or materials project with solids should be ignorant of the applications of ion beam machines and the growing expertise which surrounds them It is a pity that oversights in production and a lack of standardisation of diagrams detract slightly from an otherwise well conceived book.

N. E. W. Hartley

Spin labelling

Spin Labelling: Theory and Applications. (Molecular Biology: An International Series of Monographs and Textbooks.) Edited by Lawrence J. Berliner. Pp. xiii+592. (Academic: New York and London, January 1976.) $49.50; £25.55.

SPIN labelling is an obviously attractive technique. It is relatively easy to attach a spin label to a protein or to insert it into a membrane to act as a reporter group. Furthermore, the electron spin resonance spectra obtained are often aesthetically very appealing. But as with much modern art, the apparent surface simplicity is said to hide a deep inner meaning, which can only be properly discerned by the educated eye. In this book, a number of dedicated spin labellers try, successfully, to pass on the secrets of their art.

Probably the most useful part of the book for the experimentalist is the second half, in which applications of the technique are discussed. Four chapters are concerned with membrane studies, a reflection of the fact that it is in the field of membranology that spin labelling has proved most fruitful. Particularly useful are discussions by J. Seelig, O H. Griffith and P. Jost on the measurement of spin label order parameters, the overall conclusion being that simple measurements taken directly from the spectra usually give as good results as complex computations.

Two chapters deal with enzyme studies, but it is clear that here spin labelling has, in general, been rather less successful, in spite of the synthesis of a wide range of spin labelled compounds. The technique can be used to obtain distance information, but this is of course, information about the distance to an >N—O group rather than to a part of the native enzyme. Most enzyme studies have, however, been of a much more qualitative nature, merely looking for changes in the spectrum following some perturbation of the system.

A further two very useful chapters provide details for the preparations of spin labelled compounds and discuss instrumental aspects of obtaining electron spin resonance spectra. The first part of the book is the theoretical section containing chapters on general magnetic resonance theory by P. L. Nordio, on the theory of slow tumbling of spin labels by J. H. Freed and on the theory of biradicals by G. R. Luckhurst.

The only criticism that can be levelled at the book is that it does not meet its claim to be accessible to readers from medical and biological backgrounds. A quick survey of my medical colleagues showed that few knew what quantum mechanical operators or expectation values were, and such knowledge is assumed at the beginning of chapter two. It is a pity that the book does not include a non-mathematical discussion of the basis of electron spin resonance, since, in fact, the latter chapters show that detailed understanding of the theory is not necessary for its application to biological problems. Nevertheless, this is certain to become the spin labellers' benchside book, even if they do have to steal the library copy. **A. G. Lee**

Stimulus for ecologists

Ecological Animal Parasitology. By C. R. Kennedy. Pp. ix+163. (Blackwell Scientific: Oxford and London, 1975.) £3.50.

IN recent years a number of excellent books have been published on the general ecology and population biology of animal species with approaches ranging from the completely theoretical to practical treatments of field study problems. The ecology of two particular groups of organisms—protozoan and helminth parasites—has, however, received very little attention in general ecological texts, host–parasite relationships often being briefly dismissed by reference to the population equations of Lotka and Volterra.

Such treatment seems surprising, particularly when considered in relation to the economic importance of many host–parasite interactions. A careful examination of the parasitological literature reveals, however, that our knowledge of the ecology of the majority of parasitic organisms is very limited indeed. This unhappy state of affairs has undoubtedly arisen as a result of the complexity of many parasite life cycles, which may involve more than one species of host and many distinct parasite populations compounded by the difficulties in measurement and observation created by the intimacy of the relationship between host and parasite.

Clive Kennedy's new book is a welcome addition to the sparse literature on ecological aspects of parasitolgy. After a brief and perhaps rather superficial introductory chapter on the nature of parasitism, parasite population growth and host–parasite systems, the following four chapters are concerned with the concepts of host specificity and intra- and interspecific competition, the biology of host location and the dispersal and fecundity of parasites. Four of the remaining five chapters, constituting the major part of the book, deal in a fair amount of detail with the population biology of parasites within intermediate hosts, and poikilothermic and homiothermic definitive hosts. The contents of these chapters, reflect to a large extent the information available in the research literature, although they are somewhat biased towards the author's own interests in helminths and in particular parasites of fish. In the last chapter, perhaps the most stimulating of the book, the author attempts to come to terms with the dynamic aspects of the relationship between host and parasite populations. Some of the more quantitative attempts to model the population dynamics of host–parasite interactions are discussed, although it seems surprising that the pioneering and highly relevant work of V. A. Kostitzin 40 years ago is ignored

This book will be of more general interest to students of parasitology than modern day ecologists since the author has not really achieved his stated objective of interpreting animal parasitology in the light of current ecological ideas and concepts. For example, many rather important ecological topics such as the relationship between stability and complexity in host–parasite systems and the importance of time delays and genetic factors in population interactions are completely ignored. Furthermore, the author himself seems to be rather confused by certain ecological concepts, such as the role played by density-dependent factors in the regulation of population growth. He frequently refers to specific types of host–parasite interactions as "fundamentally unstable". The reader is first of all unaware of what the author means by stability and in addition is left to wonder how such interactions persist in the world today in the absence of regulatory mechanisms operating on population growth. In certain cases confusion is created by contradictory statements within the same paragraph. The author implying in one instant that mortality of parasites within poikilothermic hosts is a non-seasonal parameter and then going on to state that temperature influences the rate of loss of adult parasites from the host.

Many of the confusing discussions within this book are no doubt due to the lack of quantitative ecological information available in the parasitological literature. As an undergraduate text this book will be of value, and it may stimulate some of the more quantitatively minded ecologists to turn their attention to this fascinating field

R. M. Anderson

Talking to computers

Conceptual Information Processing (Fundamental Studies in Computer Science, Volume 3.) By R. C. Schank. Pp. viii+374. (North-Holland: Amsterdam and Oxford, 1975.) Dfl.75; $31.25.

How can we get computers to understand natural language? One way is to restrict conversation to a very limited domain such as noughts-and-crosses or the world of children's blocks and then to try to devise programs that will go through most of the mental operations that human beings perform when they talk to each other about such topics. In spite of some promising developments, the disadvantages of this approach are obvious: change an aspect of the simulated world—introduce a baby to play with the blocks—and sooner or later the semantic machinery will be unable to cope. An alternative method is to restrict not the topic of discourse but what the programs do. Mechanical translation, the white hope that became a white elephant, was a lesson in the dangers of an extreme version of this approach. Nevertheless, Roger Schank has opted for a wide range of discourse at the cost of limited comprehension, and the present book describes his work in great detail down to three chapters by his students based on their PhD theses. They have contrived some ingenious programs that will generate a list of paraphrases of a sentence or draw plausible inferences from it, for example, given: "John said he killed himself", their system responds: "Dead people can't talk." The drawback is, of course, that the programs are not really demonstrating a full mastery of language. They could not describe a dead man even if they had eyes to see.

The choice at present seems to be either articulate conversations with an idiot savant who knows only how to pile up wooden blocks, or the inferential responses of a deaf, dumb and blind kid who cannot even play a pinball machine. Neither alternative promises to deliver us an intelligent talking computer by the year 2001. Perhaps we should treat computers like babies and provide them with senses and voices, the ability to learn how to represent the world and to reflect on their own processes, and then talk to them for a few years **P. N. Johnson-Laird**

Manipulating plant growth

Environment and the Experimental Control of Plant Growth. (Experimental Botany: An International Series of Monographs.) By R. J. Downs and H. Hellmers. Pp. vii+145 (Academic: London and New York, November 1975.) £4.50; $12.50.

MANIPULATING the environment is a technique used by experimental and applied botanists for many years in their search towards an understanding of plant growth, and the use of sophisticated controlled environment facilities has recently increased. The authors of this book have drawn on their considerable experience with such facilities to provide a monograph that will be welcomed by many researchers in the field. Their object was to collate information on environmental factors controlling plant growth and the methods of controlling these factors in turn, to enable biologists to understand what is required in the design and use of the facilities, in order to obtain acceptable degrees of control. In doing so, the authors have produced the first major book on the subject in over ten years since L. T. Evans' *Environmental Control of Plant Growth.*

There are chapters covering temperature, light, the gaseous environment, and water and nutrients. Each begins with definitions, followed by methods of measurement and design of facilities, and examples of the influence that each factor has on the development and growth of plants. The technical information is presented with clarity. The physiological data is necessarily rather limited, but each chapter is concluded by a comprehensive reference list. The value of the book lies in its considerable amount of practical advice based on the authors' experiences, with emphasis on the problems that may be encountered. The book is concluded by chapters discussing the controlled environment in relation to the natural environment, and the various types of facilities available. The authors are directors of the South Eastern Plant Environment Laboratories in North Carolina, and naturally stress the advantages of phytotrons since a number of facilities are available and visiting workers are encouraged there. Their book will, however, prove especially useful to those with more limited facilities and little experience. **S. M. Thomas**

Look—no maths

Electrons in Metals: An Introduction to Modern Topics. By C. M. Hurd. Pp. ix+331. (Wiley-Interscience: New York and London, December 1975.) £9.95; $19.90.

DR HURD'S book is an ambitious project—the descriptive treatment of the major properties of electrons in metals without the use of mathematics. The author sets out to lead beginners in the subject through the tangled web of quasiparticles, Fermi surfaces and Kondo effects to gain at least a qualitative overview of the theory of metals. To do this the author has developed what would, at first sight, seem to be a logical framework, in that he starts with an atomistic view so as to relate the various types of metals to the periodic table. He goes on, for what is the largest section of the book, to treat the electrons as a fluid with increasing complexity, from the single-particle model to Bose condensation and superconductivity, before introducing the atoms back into the problem for a general review of cohesion and electron transport in metals.

A very large part of metal physics is covered at a fairly elementary level but this book suffers, however, from a number of major faults, which are inherent in this format. If one is to have a qualitative discussion of fairly sophisticated concepts it is necessary to provide a framework as simply and as early as possible. In the standard textbooks this would be achieved by either reference to the interplay between experiment and theory, which led to those concepts, or through a sufficiently simple model of a real metal that could be modified as one progressed in understanding. Neither of these has been followed by the present author and the simple single-particle model, on which the major part of the understanding of the electronic theory of metals can be based, is left until the final chapter. This means that many of the concepts, and indeed technical jargon, receive only the briefest mention on their initial appearance and the reader is constantly being referred forward to the later chapters. This would not necessarily be fateful, but coupled with the emphasis in large parts of the earlier parts of the book on the exception rather than the rule (as in, for instance, assymmetric scattering of electrons by impurities) must lead to terrible confusion for the beginner.

In general, although it does contain some very good non-mathematical descriptions of complex phenomena, this is not a book I would recommend for anyone without a good grounding in solid-state physics. **J. C. Inkson**

obituary

Dorothy Wrinch was born in Rosario, Argentina, on September 13, 1894 and died very recently on February 17, 1976 at Falmouth Hospital (Massachussetts). She was a brilliant and controversial figure who played a part in the beginnings of much of present research in molecular biology.

Her father was Hugh Edward Hart Wrinch, an engineer, and her mother, Ada Soutar. She was educated at Surbiton High School and won a scholarship to Girton College, Cambridge, where she read mathematics—she was a Wrangler in 1916—and moral sciences. She was appointed to a lectureship in pure mathematics at University College, London, in 1918 and became the first woman ever to receive a D.Sc. from Oxford (1929). In the early 1920s she was one of a brilliant circle of mathematicians and physicists, among her friends were Dora Black and Bertrand Russell, whom she introduced to each other. In 1922, she married John William Nicholson, FRS, then a fellow of Balliol College, Oxford, and well known for his work on atomic structure. The marriage became more and more unhappy as his mental state deteriorated; it was finally dissolved in 1937 leaving Dorothy with one daughter, Pamela. From 1922 until 1939 Dorothy Wrinch lived mainly in Oxford, holding various research fellowships and a lectureship and tutorship in mathematics at Lady Margaret Hall and Somerville College. In 1939 she took Pamela to America where she met and married, very happily, Otto Glaser, Professor of Biology at Amherst, and spent the rest of her life in the USA, holding appointments in mathematics and physics at Amherst, Smith and Mount Holyoake until she retired to Woods Hole, which she greatly loved.

Until 1932, Dorothy Wrinch's research work was all in the field of pure mathematics, particularly classical analysis. In 1930, she turned towards biology and set herself seriously to study the facts of elementary biology and chemistry by attending courses in Vienna and Paris during her sabbatical terms. She then began to seek for mathematical solutions to the problems she found, particularly of the structure of chromosomes, including nucleic acids and proteins. It was the properties of protein films that first suggested to her that peptide chains should be polymerised into sheets, by links between CO and NH groups to which she gave the name 'cyclol'. Folding the sheets then gave her a beautiful series of closed octahedra and other solid figures, built of definite numbers, *n*, of amino acid residues; one series has the general formula $72n^2$. These provided an attractive explanation of many recent observations on proteins, for example, the limited number of sizes of protein molecules found by Svedberg's early ultracentrifuge measurements. When Bergmann and Niemann in 1937 deduced from chemical analysis that egg albumin had 288 residues in the molecule, the exact number required for the second member of the above series, 72×2^2, Dorothy Wrinch felt her hypothesis had been strikingly verified. The coincidence was extraordinary. Her infectious enthusiasm carried many with her: almost all the participants of the first Cold Spring Harbour Symposium on proteins in 1938 and, most notably, Irving Langmuir Her explanation of the first X-ray evidence on the structure of insulin positively encouraged others to take up the study of protein crystal structures, W. L. Bragg and David Harker among them. (Bragg used her version of the insulin Patterson maps as one of the illustrations in his last book.)

Gradually and relentlessly, after the war, chemical and crystallographic work proved that protein molecules did not have the cyclol structure. Yet many of Dorothy Wrinch's geometrical instincts and deductions were, in general terms, correct. The structures of protein molecules are *not* based on simple parallel arrays of peptide chains, yet the packing of molecules within the insulin unit cell *is* octahedral in character, there are patterns of close packed units over closed surfaces in many spherical viruses, even the 'cyclol' link itself sometimes exists, for example in the ergot alkaloids. This pleased her—she found it very hard to give up the cyclol theory as a whole. She maintained to the end of her life a strong interest in crystallography and in biology; the book she wrote at the request of her crystallographer friends on *Fourier Transforms and Structure Factors* is still very useful. And in old age she came again to Oxford to talk over the structure of insulin.

I like to think of her as she was when first I knew her, gay, enthusiastic and adventurous, courageous in the face of much misfortune, and very kind. **Dorothy Crowfoot Hodgkin**

Dorothy Wrinch's early work was in pure mathematics, with occasional excursions into philosophy. She helped with the proofs of the 1920 edition of Whittaker and Watson's *Modern Analysis*. She wrote papers on mathematical logic in the tradition of Whitehead and Russell and, in the early 1920s, a number of papers on hypergeometric functions and problems in classical mechanics. She and I published four joint papers in the Philosophical Magazine on the theory of scientific method. I should like to put on record my appreciation of the substantial contribution she made to this work, which is the basis of all my later work on scientific inference.

We also had a joint paper on the seismic waves from the Oppau explosion of 1922, which was the first co-ordination of seismic data with the structure of the upper layers inferred from geological evidence by Suess.

Harold Jeffreys

Reports and publications

United States Department of the Interior· Geological Survey Bulletin 1395–C: Stratigraphic Nomenclature of the Thirtynine Mile Volcanic Field, Central Colorado By Rudy C. Epis and Charles E Chapin. Pp. iii+23 (Washington, DC Government Printing Office, 1974) 50 cents. [191

Airports and the Environment Pp 290 (Paris· OECD; London HMSO, 1975) 40 francs, £4 40, $10 [191

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nature

April 15, 1976

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Every cow carries a government health warning

Coronary heart disease (CHD) is a major cause of death in middle and old age in the UK and many other western countries. For instance, in the age range 45–54 years, 39% of all male deaths and 12% of all female deaths are from CHD. Since 1950 death rates from CHD have increased consistently for men; the death rate is 60% higher in that same age range now than it was 25 years ago. Strangely, there is no evidence of a similar trend for women.

In the light of these and similar figures many countries have looked at ways in which the incidence of CHD might be reduced. A working party of the Royal College of Physicians of London and the British Cardiac Society has just issued the latest assessment, which will be distributed widely to general practitioners (*Journal of the Royal College of Physicians*, **10,** 213–275). It deserves to be read by scientists too, not just for the factual content but also as an example of a rather good blend of science and commonsense.

The report comes out unambiguously against major investment in more after-the-fact services which "cannot hope to achieve a major reduction in the overall burden of CHD in the community". Preventive measures against a disease with palpable environmental factors are, however, spelt out in considerable detail. They include stopping smoking, monitoring blood pressure whenever possible, changing diet and increasing physical activity (including "getting breathless some time every day"). Probably the most controversial of these suggestions, all of which have been floating around for a long time, is the fairly explicit talk of dietary adjustments. The recommendations are made, the report says, because plasma lipid levels in the UK indicate a population generally at risk.

Fat in the diet, it is recommended, should be reduced from its present average contribution of 42% of the total calorie intake to around 35%. Saturated fats (which at present constitute half of this contribution) should be particularly reduced, to be replaced in part by polyunsaturated fats. What this means is less meat and egg yolks, more poultry and fish; less butter, more soft margarine high in polyunsaturated fats; corn oil, sunflower oil and safflower oil, not hard margarines and lard; cream and top of the milk are to be avoided, vegetables and fruit encouraged. In fact all that one's instincts say, except in the case of dairy products.

Readers from overseas, particularly from the United States and Australia, can be forgiven a certain wry amusement at this point that at last the UK is starting to publicise what has been common knowledge for many years. The word "polyunsaturated", surely the most scientific word the layman will ever need to take into his vocabulary, was sufficiently well know as early as 1970 for the Massachusetts Heart Association to be distributing leaflets to all homes asking: "Will your children remember your tasty meals, low in saturated fats and cholesterol?"

Why has it not been possible for similar exhortations (perhaps a touch less ham-fisted) to be made in Britain, and why has a major manufacturer of polyunsaturated margarine had till recently to pursue publicity for his product by unconventional means? (Not, it should be said, that it took more than a day after the report's appearance before perfectly explicit advertisements were appearing.)

Part of the answer may be in genuine scientific doubt, not so much about the role of unsaturated fats in raising plasma cholesterol levels, nor even about the correlation between cholesterol levels and the risk of CHD, but concerning the value of lowering plasma lipid levels by dietary change in trying to reduce CHD. The report itself cautiously concludes that there is "probably sufficient evidence . . . to make dietary recommendations in the hope of affecting a degree of prevention". Not, it must be admitted, the most gripping of cases.

But it is not the medical science so much as the sacred cow that has held things up. The dairy industry could be hit hard by dietary changes if the nation took them seriously—less meat, less butter, little cream. Small wonder that the issue has been carefully skirted in Whitehall for as long as possible, and small wonder that the National Dairy Council was quickly out fighting.

The publication of this report must not herald the start of some form of public relations war between rival products each of which will doubtless (such is the nature of the scientific literature) be able to marshal cast-iron evidence for its own point of view. The onus is on the Department of Health and Social Security to ensure that knowledge is diffused into the community rather than thrown at the citizen by interested parties. What is needed is a clear statement of the meaning of the scientific words and a relaively low-key assessment of the risks, followed by a requirement that advertisers state the contents of their product simply. □

Taking the naturalness out of natural disasters

Phil O'Keefe, Ken Westgate and **Ben Wisner** argue the case that disasters are more a consequence of socio-economic than natural factors

THE MEDIA continually present us with graphic accounts of natural disasters—the Bangladesh cyclone, the Nicaraguan earthquake and the African drought are just some of the recent examples of catastrophes that caused much death and destruction. Since the beginning of 1976, we have already had detailed accounts of floods in Venezuela, Australia and Indonesia, famine in Niger, landslides in Ecuador, drought in Malaysia and the mammoth earthquake in Guatemala. It is difficult to gather global information on the frequency and, more importantly, the impact of these disasters. But a set of global statistics from all available resources has recently been compiled.

The Disaster Research Unit at the University of Bradford has collected data from international organisations, government departments, academic institutions and insurance companies. The information was confused because most institutions recording disaster data had an implicit role in the disasters and their aftermath which coloured their recording. For example, the international and governmental organisations were chiefly concerned with disasters in which aid was being donated, academic institutions were primarily interested in recording unusual phenomena which might not necessarily be disastrous, while insurance companies only recorded information directly related to their business. In spite of the data's unreliability, several tendencies can be observed.

The most important tendency is an increase in the occurrence of disasters over the last 50 years. Figure 1 shows this increase from 1947–70 of large-scale disasters, that is, those disasters covering more than a ten-degree square on a world map and where damage exceeds $1 million. This tendency is paralleled by an increasing loss of life per disaster. The greatest loss of life per disaster is observed in underdeveloped countries, and there are general indications that the vulnerability of these countries in particular is increasing. Such conclusions clearly require further explanation.

Disaster marks the interface between an extreme physical phenomenon and a vulnerable human population. It is of paramount importance to recognise both of these elements. Without people there is no disaster. The two elements are basic to an explanation of an increase in the occurrence of disasters. No major geological or climatological changes over the last 50 years adequately explain the rise. There is little argument about geological change, but there has been much mystifying argument about climatic change, especially following the prolonged drought over the African and Asian continents. But no firm conclusion can be drawn about changing climatic conditions from available evidence. Randall Baker at the Development Studies School of the University of East Anglia recently reviewed all the evidence of climatic change in Africa, and offered the Scottish judgment of "case not proven". Even if some long-term change was observable it would not explain the increase in disaster occurrence observed in the data.

If it is accepted that there has been no major geological and climatological change in recent years, then it can be assumed that the probability of the extreme physical occurrence is constant. If the probability is constant, then logically the explanation of the increasing numbers of disasters must be sought in an explanation of the growing vulnerability of the population to extreme physical events. Ongoing research suggests that some radical rethinking on the nature of "natural" disasters is necessary.

Photo: AP

Searching for water in Ethiopia.

It is known that the frequency of natural disasters is increasing especially in underdeveloped countries. Indeed, the increased vulnerability of people to extreme physical events can be seen as intimately connected with the continuing process of underdevelopment recorded throughout the world. As population continues to expand, and as resources continue to be controlled by a minority, the real standard of living drops for much of the world's population. This population is increasingly vulnerable to environmental variation as the process continues. Paul Richards of the Environmental Unit in the International African Institute recently emphasised this in his introduction to *African Environment: Problems and Perspectives:* " . . . just as natural processes such as lack of rainfall affect social structures", he argued, "so social processes such as economic 'development' can affect natural systems, 'causing' famine and soil erosion for example". He went on:

> "[I]n a continent where international ties of dependency, massive international labour migration and multinational companies prevail and in a world where growth does not necessarily mean development, and development does not necessarily bring enrichment or an increase in personal happiness, the ultimate cause of environmental problems may well be traceable to the structural imbalances between rich and poor countries and we would be right to replace the term *natural* with the more appropriate term *social* or *political* disaster".

These suggestions would strike the Guatemalan peasant as commonsense. The recent earthquake there is no longer identified as a natural event—the local inhabitants who survived are referring to the event as a "classquake". It is a view which reflects their broad experience. That experience is the basis for an explanation of their general plight in terms of a process, not of development, but of underdevelopment —a process which their increasing vulnerability reflects. Instead of moving independently (as one school of thought argues) from a state of undevelopment to development along the lines that the now-developed west has already done, Third World countries (says an alternative, radically opposed school) have in fact been moving retrogressively from a state of undevelopment to one of underdevelopment, in a process of "marginalisation" which is not so much separate from as the price of the west's development in an increasingly inter-

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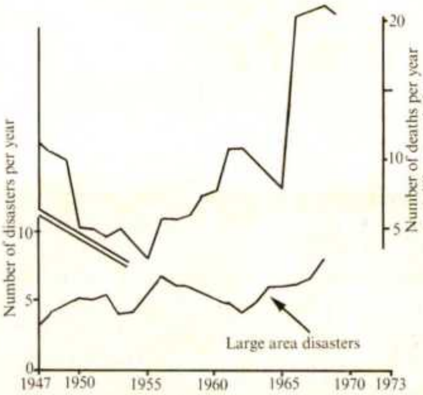

Fig. 1 Global disasters 1947–1973: Five-year moving average.

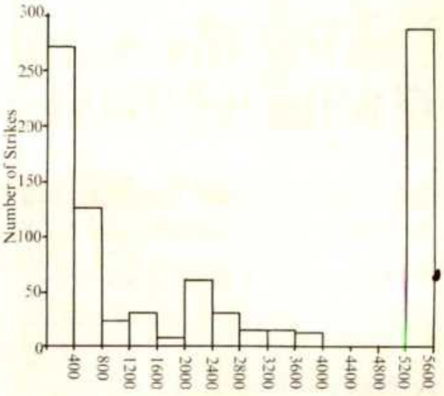

Fig. 2 Number of disaster strikes by per capita income of disaster-strike area: the large number of disasters recorded in developed countries is a reflection of the closer monitoring of disaster events.

dependent world. As this has happened, the relatively deprived sectors of the populations within these countries have become even worse off.

The reality of this situation is shown in Figure 2. It is the poorest countries which tend to suffer most disaster strikes. This point ought to encourage precautionary planning to mitigate the effect of future disaster. Such precautionary planning needs to be totally integrated into planning for real development, which means the necessary concentration on the vulnerability of a population to future disaster can only be done successfully through an understanding of the marginalisation process. Emphasis on precautionary planning does not, of course, make unnecessary the valuable action invariably mounted in the aftermath of a disaster: in fact, the formation of the London Technical Group, which supplies accurate technical reports on the position after disasters and generally provides expertise on relief measures, is to be warmly welcomed. In the long run, however, precautionary planning would be more beneficial than relief work, since it would aim to consider and alleviate the causes and not merely the symptoms of disaster.

Successful precautionary planning, then. in focusing on the population's vulnerability, depends upon the identification of cultural attitudes towards the use of indigenous resources at local and regional levels, and the incorporation into development planning of strategies to mitigate disasters; precautionary planning should be seen as the insurance chapter in any development plan. The aim would be to raise the standards of life of people currently ill-placed to resist disasters because those standards are too low. The average "cost" of a disaster strike, a rather meaningless concept when the range of impact is so great, is about $20 million. It is sufficient to say that more than $1,100 million was spent on disaster assistance in 1973; and 96 countries of the world had less than this amount of resource capital as GNP in 1973. The time is ripe for some form of precautionary planning which considers vulnerabliity of the population as the real cause of disaster —a vulnerability that is induced by socio-economic conditions that can be modified by man, and is not just an act of God. Precautionary planning must commence with the removal of concepts of naturalness from natural disasters. □

After the Guatemalan earthquake in February—and there are suggestions of another disaster to come with the onset of the rains this month. (Photo: AP)

High technology protectionism

Colin Norman reports from Washington on US efforts to control the export trade in strategic technology being stimulated by the era of "détente"

A REPORT which has so far received scant public attention, but which is now being closely studied in the Pentagon, contains a number of startling and disturbing recommendations for controlling trade in high technology goods between western nations and the Soviet Union. Prominent among them is the suggestion that the United States should apply tough sanctions against any country—including America's European allies—which passes potentially strategic technology to a communist country. It also recommends that the Department of Defense should keep a close watch on government-to-government scientific exchange agreements and that it should keep its eye on the training of people from communist countries at major institutes and universities in the United States.

The report, published by the Defense Science Board (DSB), a top-level Pentagon advisory group, is the latest in a series of outpourings of concern that the United States' lead over the Soviet Union in strategic areas of technology is gradually being whittled away by commercial sales of computers, electronic goods and similar items. Such trading has mushroomed in the past few years through the establishment of the Nixon-Kissinger policy of detente with the Soviet Union.

The DSB report, prepared at the request of senior Pentagon officials and written by a task force headed by J. Fred Bucy, vice-president of Texas Instruments, argues that national and international arrangements for controlling east–west trade have not been effective in preventing strategic technologies from leaking eastwards through commercial channels. It therefore argues for a new approach, including some harsh sanctions to prevent such transactions.

Trade between the United States and communist countries has been controlled since the beginning of the cold war by a mechanism operated by an office in the Department of Commerce called the Office of Export Administration (OEA). A company wishing to export a strategically sensitive product or technology, as defined in a document known as the Commodity Control List, must apply to OEA for a licence. The application is reviewed by an interagency committee composed of representatives from the Departments of State, Defense, Commerce, the Energy Research and Development Administration, and the CIA. Approval is given only to those transactions deemed unlikely to improve the Soviet Union's military capabilities.

A measure of western conformity in such matters is provided by an arrangement known as the Consultative Group Coordinating Committee (CoCom), consisting of representatives from NATO member countries, excluding Iceland but with the addition of Japan. CoCom maintains a list of restricted products similar to the US Control List, and its members have informally agreed to follow similar procedures in licensing exports to communist countries.

Although both the US Commodity Control List and the CoCom list have recently been brought up to date—during the height of the cold war they were said to contain such strategic items as brassieres and wigs—the DSB task force suggests that they still contain many products of little military value. Instead of wasting time on such items, the task force suggests that the list should be drastically shortened and more effort should be put into curtailing the transfer of vital design and manufacturing knowledge. Particularly close attention, it indicates, should be paid to deals involving the transfer of turnkey factories, products requiring sophisticated maintenance and operating information, and items resulting from a revolutionary technological advance.

The report argues that controls have broken down in recent years because "CoCom members have perceived less of a need to maintain strict controls while the opportunity for individual gain through the sale of technology to communist countries has increased. As a result, strategic technology has been transferred to communist nations through CoCom-sanctioned exceptions, ambiguous interpretations of lists, and, perhaps, conscious violation of CoCom agreements". The task force therefore suggests that the United States should try to bully CoCom countries into adhering to export control agreements by imposing "a sanction on any CoCom country that fails to control a specific technology, by restricting the flow of know-how in that technology to the offending country".

Another reason why controls have broken down, the task force argues, is that firms in some countries which import US technology promptly re-export it to the Soviet Union, in violation of international agreements. Though the United States already attempts to stop such transactions by blacklisting firms thought to be engaging in such practices, the task force argues that some strong-arm tactics are in order here, too. "A nation that allows strategic technology to be passed on to a communist country should be restricted from receiving further technology of US origin", it states.

Moreover, the task force suggests that the United States "should release to non-allied, non-communist countries only the technology we would be willing to transfer to communist countries directly". In that regard, the task force states that "of particular concern is the acquisition of high technology know-how by nations of the middle east, and the assimilation of know-how by nations of Western Europe that are not members of CoCom—principally Switzerland, Sweden and Austria".

Though the bulk of the report deals with transfer of strategic technology through commercial channels, it also notes that recent scientific exchange agreements between the United States and the Soviet Union have greatly increased the movement back and forth of scientists between those two countries. Such agreements, Bucy notes in a covering letter to the report, are potentially "an area of concern".

The task force, finally, comes up with a recommendation that the Department of Defence should conduct a "comprehensive study of active mechanisms for transferring technology that are beyond the normal scrutiny of export control administration", and that it should develop recommendations for "monitoring and controlling them". Among such mechanisms, the task force lists "the use of US citizens as consultants for key technologies by communist countries", "the participation of US citizens as principals in firms established outside the US and engaged in transferring embargoed technology and products to communist nations", and "the training of citizens from communist countries at the more significant laboratories of US technical institutes and universities".

The task force report was approved for publication by Malcolm Currie, Director of Defense Research and Engineering, which suggests that it at least has his general approval. An internal task force has now been established in the Pentagon to evaluate the report and to recommend if, and how, its recommendations should be implemented. ☐

USSR

Conditions on educational success

The Soviet Union's Five-Year Plan for 1976–1980 will make increasing demands upon the higher educational system. Vera Rich looks at what new degree regulations being introduced could mean for Soviet science

By 1980 the Soviet economy will require 9.6 million "specialists" and 11 million persons with higher educational qualifications in order to attain the envisaged levels of economic growth and technological expansion. Hardly surprising, then, that considerable attention is being paid to problems of streamlining and improving the Soviet education system, particularly in the higher echelons; but a review of the method of awarding higher degrees is producing changes which can only be regarded as worrying.

The degrees of Candidate of Science (roughly equivalent to PhD) and Doctor of Science are not awarded by the Universities, but the Ministry of Education; Universities and Research Institutes make the necessary recommendations, but the confirmation is by no means automatic. The body which assesses the recommendations and awards degrees is the Higher Qualifications Commission (*Vysshaya Attestatsionnaya Kommissiya*, VAK) of the Ministry.

On January 1 of this year, however, new regulations came into force, introducing a major reorganisation in both the structure of VAK and its operation which must, inevitably, reflect on the whole structure of Soviet research and the academic establishment. According to Professor V. G. Kirillov-Ugryumov, the Chairman of VAK, in a recent *Pravda* interview, these changes are not merely of an administrative nature; they also have a qualitative significance. The status and "potentialities" of VAK have been extended. Requirements have been raised regarding the level of qualifications and, consequently, on entry into a scientific career.

In its reorganised form, the administration of VAK will be in the hands of a Plenum, a Presidium and a "College", in whose work leading scientists and specialists will participate. There are to be 34 councils of experts dealing with the principal lines of research, and also some 500 "specialised councils" for assessing degree theses, 150 of these councils being entitled to deal with Doctoral degrees. The Presidium and "College" of VAK have been operating since May 1975, and the first session of the Plenum met last July, but the establishment of the councils is not yet complete, although it is hoped to have them all fully organised by June of this year.

Regarding the "qualitative" changes, the new regulations, says Professor Kirillov-Ugryumov, will demand a higher standard in the theses submitted, and the postulants themselves will also have to meet certain more stringent requirements. The new regulations require that they should "combine a profound professional knowledge with a mastery of Marxist-Leninist theory, and with the convictions of an active builder of communist society".

Courses in political theory and participation in practical "community activity" have always formed a *de facto* compulsory part of Soviet higher education, but the student body and even the staff have tended to regard them as a necessary chore demanding little more than their physical presence. Hitherto the Soviet educational system, although Party-orientated, has permitted a certain number of non-Party scientists to rise to considerable academic heights (Academician Sakharov being the most notable example). The new stress on the political outlook for higher degree postulants, in which their "community and political activity" as well as their scientific knowledge is taken into consideration, and the presence of Party and trade-union representatives on the "specialised councils", suggest that these days may be over, and that the non-Party element in science will gradually be phased out.

Significant, too, is the fact that almost all Professor Kirillov-Ugryumov's comments on the "distinguishing features" of the new regulations deal with these political aspects, on the academic side he notes merely that all exemptions from the examinations for the degree of Candidate and other "weaknesses" of the existing system will be eliminated, and that a higher standard will be demanded from the "official opponents" in the formal defence of doctoral theses.

Although the new regulations had not been published at the time of their introduction (a booklet of the full text is promised), their content and implications were well-known in Soviet academic circles some months in advance. As early as August 1975, reports from dissident sources were affirming that, under the new system, from 1985 onwards no non-Party member would be able to hold a major academic or scientific post, so that students wishing for a successful career in science would be well-advised to take steps to enter the Party ranks (via the Komsomols) at their earliest opportunity.

Politically speaking, the new emphasis on *partiinost'* does make a certain amount of sense—it was precisely the participation by the non-Party scientists that gave the dissident movement its real strength and significance. Scientifically speaking, however,. while the new regulations may, in Professor Kirillov-Ugryumov's words, reflect a "qualitative" change, historical parallels would suggest that it is one which, in the long run, could be detrimental to Soviet science as a whole. ☐

ITALY

Trieste still troubled

Gillian Boucher reports from Trieste on the threat facing an international institution supported by funds from diverse sources

The International Centre for Theoretical Physics at Trieste is threatened with a serious cut in funds which, if not compensated by money from other sources, could result in its closure. What is in jeopardy is the contribution from the United Nations Development Programme (UNDP), which was to have provided nearly one-fifth of the 1976 budget of $1.2 million. UNDP provides funds for specific scientific activities: the regular programmes on solid state physics and applied mathematics. Since an irreducible 35% of the total budget goes on overheads, cutting out UNDP support would mean an enormous reduction in the scientific work of the centre.

Disaster almost struck just before Christmas when the centre received a cable from UNDP announcing that no money would be available for the course (then due to start in three weeks' time) on the interaction of radiation with condensed matter, and that the sum for the 1976 mathematics course would be halved. Fortunately the centre had in writing UNDP's previous promise to support these courses, and managed to persuade UNDP that it was impossible to cancel the solid state course. Funding for the course was restored 17 days before it began, but the cuts for next autumn's mathematics course remain.

More serious is the likelihood that UNDP funds will soon dry up altogether. UNDP has been suffering badly from inflation and from the failure of some countries, notably the USA, to pay up. Its projected shortfall for 1976 of $100 million means cutting back on many projects, and Trieste is a likely victim. Trieste is also likely to be hurt by the growing proportion of UNDP funds being assigned to individual nations rather than international projects.

The centre has always found living with UNDP a precarious business: meetings and courses have to be planned two years ahead while UNDP commits itself for only a year at a time. The Swedish International Development Authority, which provides the bulk of the funds to support the centre's Associates (scientists from less developed countries who come to do research at the centre three times in five years for up to three months at a time), also votes funds annually More stable are funds from the Italian government (currently providing $350,000 a year) and the International Atomic Energy Agency (IAEA) ($225,000 a year), which votes funds on a two-yearly basis. To ease planning headaches and reduce the "rampant feeling of uncertainty" among the centre's small technical staff, an *ad hoc* consultative committee did in fact report to IAEA last summer that the unstable UNDP contribution should be taken over by the centre's stable sources of income

With luck that is what will now happen. IAEA and UNESCO have recently expressed a wish to compensate the centre if UNDP funds dry up, and are proposing to increase their funding to $450,000 and $300,000 respectively Two potential problems seem to have been overcome The first was that the IAEA contribution has been no greater than that of UNESCO since 1970, the year in which UNESCO first became a major source of funds IAEA has however now agreed that its contribution need not be fixed at the UNESCO level, and indeed that with UNESCO's current impoverishment such a rule would be absurd. The other difficulty was that UNESCO's policy has been to fund a project for no more than 10 years, after which other sources of income (primarily national governments) are supposed to have been found But there is now hope that the rule will change, as the committee studying it recently reported to UNESCO that it is outdated, a relic of the era of centres set up by regional bodies. Certainly it would be shortsighted in the extreme to apply the rule to a centre used by over 50 nations, most of them poor.

There should thus be no fundamental difficulty The centre's well-being will depend upon the attitudes of those attending the imminent executive board meetings of IAEA and UNESCO and their general conferences in September and November of this year. At Trieste there are worries particularly about Britain's representative at UNESCO, Sir Harold Thompson, who is likely to repeat his view that Trieste is eating up too large a slice of UNESCO's $1 million basic science cake, and may sway some of the Commonwealth countries. Ironically, at the same time that British scientists have provided the strongest support for Trieste and the backbone of its teaching, the British government, through the international agencies, has caused it great worries.

If the proposed increases are approved by IAEA and UNESCO the centre will be slightly better off than at present but will of course have to exist at this level of funding through the next four years' inflation. Next year is in any case bound to be a messy one because the present uncertainties about money make planning almost impossible. But this year's programme is the largest ever and it is hoped at least to maintain the present level of activity: three 3-month courses and around 1,000 scientists passing through each year. New ideas are being tried; this summer there is a science teaching programme designed in particular for those scientists whose backgrounds are so weak that they cannot benefit from the other activities of the centre. The emphasis of the centre is on science at all cost; two of the three senior administrators as well as several secretaries have recently been made redundant, with the inevitable result of reduced efficiency and some discontent among the remaining staff.

If the UNDP funds are not replaced by those from IAEA and UNESCO, the centre's director Abdus Salem would prefer it to close rather than to continue with a grotesquely reduced programme. Closure would be a tragedy. The centre educates, stimulates and boosts the morale of thousands of physicists from less developed countries, providing them with the sort of contact with their peers which any American or European scientist takes for granted. And it is really very cheap: its running costs are the equivalent of less than 1% of Britain's SRC budget. ☐

JAPAN

China energy link weakens

JAPAN'S energy policy still accords an essential role to oil, even though the crisis precipitated in 1973 showed it to be the most vulnerable of the industrialised countries because of its great dependence on oil imports. Like those countries, however, Japan is now having to cope with an emerging glut of oil, and in the last couple of months relations with one comparatively new supplier in particular appear to have changed.

The supplier is the People's Republic of China, with which the level of Japan's two-way trade last year reached a record $3,800 millions The two countries began their oil trade in 1973, and last year oil produced almost half of China's export earnings from Japan.

In January there were reports, viewed at the time rather sceptically by Japan's refiners and electricity companies, that China might buy over 60% more steel from Japan in return for Japan increasing its purchases of Chinese oil from 8 million tonnes last year to 10 million in 1976.

The following month, however, before any 1976 agreement was concluded, China advised Japan that its February shipment would be halved The same was expected for March as well, and one theory had it that supplies were being diverted to North Korea. When the first contracts were finally agreed in early March, the trend was confirmed: one of Japan's two major importing consortia agreed a 12½% cut. The other principal buyer has since contracted to take a 30% reduction. Japan's total imports of Chinese oil are thus unlikely to exceed 6.1 million tonnes, although there are options which, if exercised, would boost the total to 1975 levels.

It now appears that the February reductions merely anticipated the smaller contracts, and that the reductions themselves simply reflect Japan's oil needs at the present time (with the fact that China's oil is more expensive and less easily processed also playing a part). China may still be hoping to cash in on any upturn in Japanese demand, and might then be looking to offer longer-term oil contracts in return for the Japanese steel it now seems to want and which Japan's steel companies (shareholders in the oil importing consortia) are keen to sell. For the moment it is China's political upheavals which may preclude immediate progress on this.

IN BRIEF

Kissinger on sea law

Dr Henry Kissinger has urged the United Nations Law of the Sea Conference, now in the middle of its latest session in New York, to reach an early agreement, and has made suggestions to this end. Agreement was near on issues like the 200-mile economic zone and a 12-mile territorial limit, and on the continental shelf and protection of the marine environment, but, he said, the critical issues still to be settled concerned exploitation of deep sea beds, on which the USA's proposals were for an international sea bed resource authority, and arrangements for compulsory and impartial settlement of disputes. There was also the matter of marine research within the 200-mile zone. (The US Senate passed a bill at the end of last month to establish such a zone off its coasts; it gives fishing priority to Americans and requires foreign vessels to obtain permits.) Dr Kissinger said the various issues were linked: failure to agree on them could eventually lead to war.

Energy consumption down

Figures released in the last fortnight confirm that total energy consumption in both the United States and the United Kingdom has been falling, and for essentially the same reasons. Apart from a stimulated interest in energy conservation and the benefits of a comparatively mild winter, the chief factors responsible for the reduction in both countries have been higher energy prices and the industrial recession. The US figures reveal a 2.5% drop last year (4.8% over the last two years), while Britain experienced a 4.5% fall in the winter months of November, December and January. Oil consumption was down 5% in Europe and 1% in North America last year.

Middle East nuclear weapons

A report that Israel has an arsenal of 13 locally-made atomic bombs, decried in the Israeli press as speculation and part of a propaganda campaign by US officials favouring American arms supplies to Arab countries, provoked a response from Egypt's President Sadat last week on his visit to Italy as part of a European tour. He said he did not know if the rumours were true, but warned that Israel would have to face the consequences if it used nuclear weapons in the Middle East.

Conferring doubt

KENNETH MELLANBY

As money to support scientific research becomes scarce, it is right that we should scrutinise spending on what may possibly be fringe activities. Today a great deal which might go to pay salaries or to buy equipment is spent on organising meetings and conferences, and in paying the travelling and other expenses of those who attend. This can only be right if the scientists who attend these conferences do better research as a result. No one seems to have studied the "cost-effectiveness" of the scientific meeting.

Before the last war most of us seldom expected a grant and we went to conferences in our own countries or abroad at our own expense; if a small sum of money was available, it was received with gratitude and divided among a group of colleagues who gladly made up the balance out of their own pockets. Now most scientists expect the fullest public support, and there is even trade union pressure to insist that all shall receive the maximum government rate for the country visited, even if this is excessive, and even if poorer colleagues are denied a share of their costs. The total sum involved is substantial.

I would not deny that some conferences are fully justified; I think that they can be easily identified Thus I personally gained a great deal from a Ciba Foundation Guest Meeting convened in January 1966 by Dr T. A Quilliam of University College, London, on the apparently restricted subject of "The Mole; its adaptation to an underground environment". Some of us, all actively working with this animal, met for two days of papers and discussions. Most of the work described had not yet been published Everyone was interested in everything that was said, and all could enter fully into the extended discussions.

Another equally valuable, though slightly larger meeting was an Advanced Study Institute, sponsored by NATO, and organised by my colleague Dr N. W. Moore at Monks Wood Experimental Station in July 1965 Its subject was "Pesticides in the environment and their effects on wildlife". About 75 scientists, including the majority of those from all parts of the world who were actively researching in this field, met for a fortnight to compare notes Most of the papers dealt with unpublished material. Everyone was deeply concerned in the subject and able to contribute something useful to the discussion.

Thus it would appear that small, highly specialised meetings can be successful, and helpful to research I fear that the same cannot be said for the enormous international gatherings arranged by many learned societies. Here few papers contain anything new. Most of the speakers read a paper as a condition of receiving a grant. Many of those supported by public funds are absent from most of the sessions. Sometimes it is an opportunity for younger scientists to see and hear some of the elderly savants who may already possess legendary reputations, but unfortunately they often discover that their idols have tongues of clay.

It may be admitted that the public sessions of large conferences are unproductive, but it is suggested that the informal contacts, in the bar and elsewhere, justify the expense I cannot confirm this I have asked many colleagues whether they can honestly say that such contacts have ever had a major and productive effect on their research So far no one has given me an example. Contacts between working scientists are undoubtedly important, but large meetings with their social distractions may be the worst places for them to take place. The distractions may be fun, but why should they be enjoyed at the taxpayer's expense? The money could probably be better spent sending young and productive scientists on visits to selected laboratories ●

correspondence

Incubator contamination

SIR,—Tissue cultures usually require a CO_2-in-air incubator to maintain the proper *p*H in the medium. When chemicals are introduced into the culture to test for their carcinogenic or mutagenic activity, potentially hazardous materials might be volatilised and released through the incubator vent.

Air from an incubator (37 °C) containing four large covered dishes, each of which held three smaller covered dishes containing about 50 μg of safrole in 8 ml of medium, was drawn through a bed of activated charcoal. The charcoal was treated with CS_2, and the eluate was analysed by gas chromatography. The concentration of safrole in the gases vented from the incubator was about 2×10^{-2} parts per 10^9.

Concentrations of such magnitude may prove to be insignificant, but investigators should be aware of this possible source of contamination. A partial solution to potential exposure of personnel may be achieved by connecting the incubator vent to an exhaust system (for example, an operating chemical fume hood) by large-bore tubing. This expedient will, however, neither prevent the release of potentially contaminated air into the laboratory each time the incubator is opened, nor eliminate the possibility of transmission of volatilised material from one dish to another within the incubator.

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Angling right

SIR,—Kenneth Mellanby (March 4, page 7) is right to claim that anglers are responsible for the continued existence of the large quantity and good quality of freshwater fish in parts of the United Kingdom, particularly in England where the need to pollute is greatest.

In earlier and cleaner times, when fish were threatened by over-harvesting, 74 Angling Societies in London were the principal supporters of the Mundella Bill for the Preservation of Non-migrating Fish in England and Wales. It became law in 1878 (though not before jealous rows between the larger of the Societies and after fierce debates on whether or not to include a close season for the migrating species), and for the first time curbed the netting and marketing of "white fish", which by that time had reached wholesale proportions in the effort to feed fresh protein to a growing population.

Once refrigeration, Argentine beef and New Zealand lamb became available the pressure on freshwater food eased dramatically, and it is rare to meet anyone in this country who enjoys aromatically baked pike, grilled perch or the carp, once the favourite of the abbots. All three species are the equal of the salmon and infinitely superior to the pathetic offal-fed restaurant trout.

Today, as Mellanby emphasises, the danger is pollution in all its many and sometimes subtle guises, and it is curious how long it was before anglers organised themselves in a co-operative to spread the risk of the cost of bringing a Common Law action against interference of any kind with an established fishery. John Eastwood founded the Anglers Cooperative Association in 1948, and it has since won costs and compensation in almost a thousand cases of pollution, losing one on a technicality.

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Science indicators

SIR,—It is often asserted that science ranks with politics at the bottom end of public esteem. But the survey conducted for the National Science Foundation in the US (March 18, page 184) discerned a remarkably favourable attitude toward science and technology among Americans. The survey found that 75% of the American public believes that science and technology have changed life for the better; a similar survey conducted in 1972 discovered that only 70% then held that belief. In addition, scientists and engineers were ranked second and third respectively in terms of occupational prestige—below doctors, but above ministers of religion. Businessmen ranked lowest.

As for the impact of science and technology, there seems to be a certain amount of ambivalence. Some 57% of the respondents said they think that science and technology have done more good than harm, while 31% said they believe the beneficial and harmful impacts are evenly balanced. Only 2% believe that science and technology have done more harm than good. By far the most frequently cited benefit was improvements in medical care.

When asked whether social control over science and technology should be increased, 28% of the respondents replied that it should, while 46% said they believe the degree of control is about right.

The results of the survey also conflict sharply with the popular belief that young people are particularly turned off by science and technology—the attitudes of people under thirty differed little from those of elders.

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Mass and weight

SIR,—The introduction of the SI system of units presented a great opportunity to clarify the difference between the concepts of mass and weight. It is thus disappointing that the use of the word kilogram has already in common parlance become associated with weight rather than mass. This is illustrated in the jingle put out by the British Metrication Board which you quote in an example in Competition 6 (March 18, page 188).

Although in most cases weighing is merely a convenient way of determining mass and "weights" used with balance-type instruments are calibrated in mass units, it would have been preferable, for example, to express the contents of a jar of jam as net mass 453 g rather than net weight, as has become the custom. The widespread television coverage of space flights has well illustrated the concept of weightlessness and it should no longer be difficult even for children to appreciate the distinction between mass and weight.

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The JPU in modern science

SIR,—D. Fraser is to be congratulated for his valuable concept of the JPU (Just Publishable Unit) presented in *Nature* (Correspondence, March 11). A more conservative measurement, however, is the JXPU (Just Xeroxable Published Unit), which may be defined as the smallest amount of published information normally considered worthy of xeroxing. An example being D Fraser's correspondence.

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news and views

Ball lightning

from P. C. W. Davies

REPORTS of unusual aerial phenomena always attract great interest, are surrounded by folklore and superstition and irritate scientists who find them hard to explain. By far the most common such reports concern luminous spherical or ellipsoidal objects which appear without warning, often move through the air erratically, and disappear without trace, sometimes explosively. Each year, hundreds of eye witness accounts of such events are reported to local newspapers, the police and even military authorities. Occasionally the accounts are published in scientific books and journals, and several have appeared in *Nature* in the past few years When the events occur during adverse weather conditions they are often called ball lightning The invention of a precise name is somewhat misleading because it suggests that these phenomena are well understood. They are not. Indeed, so puzzling and bizarre are the properties attributed to these luminous balls that many scientists have preferred to deny their existence.

For the theoretical physicist, the hardest thing to swallow is that the balls are often said to be encountered inside closed, or nearly closed, structures. One spectacular report appeared a few years ago of a luminous sphere moving down the inside of an all-metal aircraft in flight. More frequently, witnesses claim to observe ball lightning inside their own homes.

One particularly alarming account of such an event is described by M. Stenhoff of Royal Holloway College, London University, in this week's issue of *Nature* (page 596). The case is particularly intriguing because the single witness, a lady living in the English Midlands, was able to produce material evidence in support of her story During the course of her unnerving experience, the witness claims she was struck full in the body by a blue sphere of light, which totally disintegrated her clothing at the centre of impact The dress she wore has a hole several centimetres across Surprisingly, she sustained little physical injury, complaining only of a burning sensation in her wedding ring when she instinctively brushed the object away.

Taken at face value, this report highlights the hazards surounding the present lack of understanding about these phenomena It is not at all clear, for example, that conventional lightning precautions are of any use against such things, or indeed that ball lightning actually has any direct connection with ordinary lightning. None of the several theories which has been proposed in recent years accounts for the properties of the luminous spheres completely satisfactorily. The most difficult part is to explain how they can remain coherent, stable and luminous for durations up to, apparently, some minutes. There is also the peculiar kinematic behaviour—in many cases the balls are said to move upwind, and even trail along beside aircraft travelling at several hundred m p.h. through the air. Electrical explanations based on ionic recombination fail to explain the long lifetimes. Various plasma theories have been published, but run into problems of stability. Even nuclear theories have been tried They would predict that the Midlands lady should be suffering from radiation sickness. Perhaps the most imaginative suggestion of all is that micrometeorites of antimatter are responsible!

One of the most promising theories is due to the Russian physicist Peter Kapitsa, who has proposed a model based on the existence of UHF electromagnetic waves produced by an ordinary lightning strike A standing wave pattern could then be created under some circumstances, which might then ionise the air in the region of an antinode, thus producing a luminous patch which could be sustained in energy by the external field. This theory certainly accounts for many of the enigmatic properties of the luminous balls, but is not completely convincing. It seems likely that some nonlinear, coherent electromagnetic process which is still not understood is regularly taking place in the atmosphere.

As always with transient aerial phenomena, scientific progress is hampered by lack of precise observational data Accounts often reflect the beliefs and perceptual distortions of the witnesses. In addition, the usual unreliability of the human memory for accurately recalling details of speeds, sizes, durations and so on makes any theorising based on random narrative evidence from the general public particularly risky.

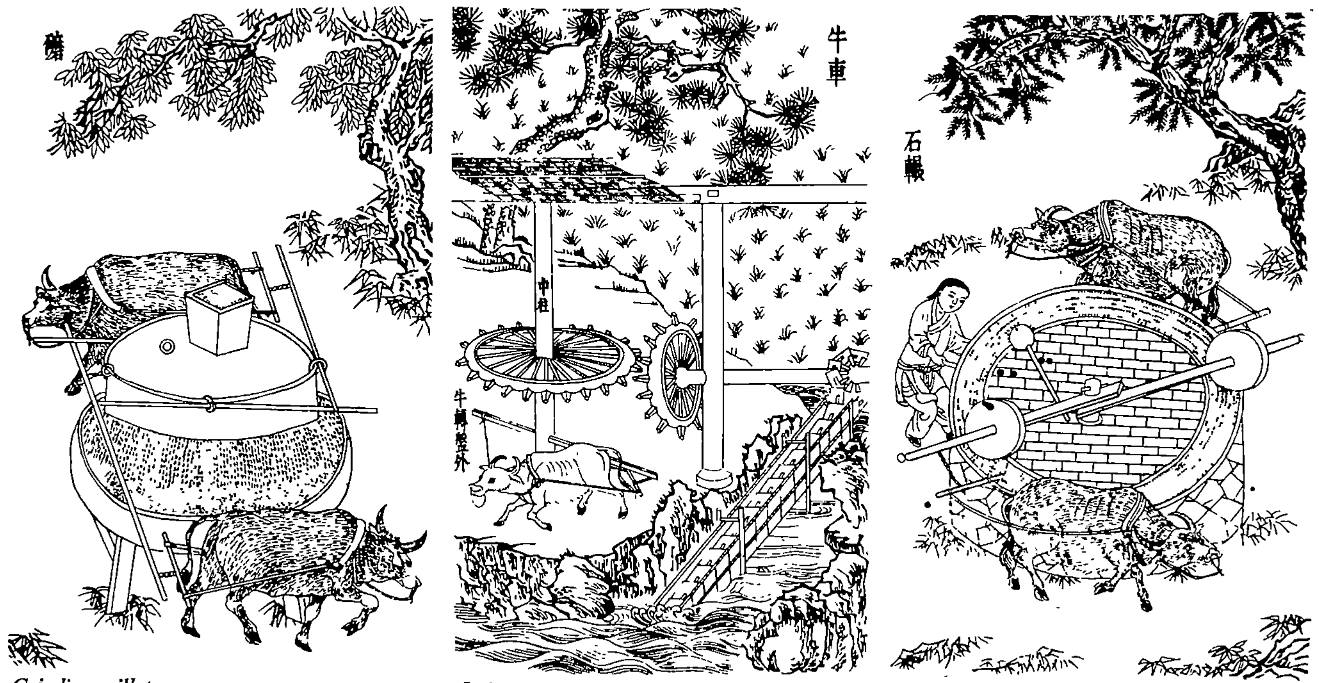
The present unsatisfactory situation would be greatly improved if the aura of mystery and superstition surrounding unusual aerial events were dispelled. Good, detailed eye-witness reports of luminous balls are frequently made by competent observers such as airline pilots, but are rarely passed onto scientists. Instead many of them find their way into military files, where they are shrouded in a ridiculous secrecy. (Incredibly, the British Ministry of Defence continues to deny scientists access to their accounts of these events.) With proper cooperation between scientists and the public, particularly the local press, and the civil aircraft authorities, it would be possible to follow up ball lightning reports rapidly, enabling tests for radioactivity and so forth to be carried out. One sensible improvement would be for the Meteorological Office to draw the public's attention to the existence of this potential hazard, particularly during stormy weather, and request detailed reports. ☐

A hundred years ago

A CORRESPONDENT, Mr. F. Green, writing from Cannes, France, states that on the 8th instant, for the first time this year, he heard the Cuckoo in a valley amongst mountains sixteen miles to the westward of that place. The first time last year that he heard it in the same neighbourhood was on the 10th of April.

ON April 2 at 5.55 a.m., an earthquake was felt at Berne. Two movements took place from east to west. The duration at was two seconds; doors were opened, and church bells were rung by the shocks. In Neufchatel a strong detonation was heard; the oscillation was very strong in the lowest part of the city, and clocks struck the hour before the appointed time. Persons who where in the streets declared that warm wind was blowing for some seconds. A few hours afterwards a rain-spout occurred near Mainz, in Rhenish Hesse. A number of houses were struck by a thunderbolt and ignated, many others were flooded by the water falling from the mountains, and people drowned by an instantaneous flood.

from *Nature,* **13,** April 13, 475; 1876.



Grinding millet or corn *Irrigation* *Milling grain*

Buffaloes in Chinese agriculture: from a treatise published in 1742. The buffalo is still used widely in China today as a draught animal. (*The Buffaloes of China*, by W. Ross Cockrill (FAO, 1976)).

Order and spacing of ribosomal RNA genes

from Benjamin Lewin

THE first system to be defined in which a large precursor RNA is processed to much smaller mature molecules was that of rRNA synthesis in eukaryotic cells; as early as 1966, Greenberg and Penman (*J. molec. Biol.*, **21**, 527–535) reported that a label in nucleolar RNA first enters a 45S species in HeLa cells and only later is found in 28S and 18S rRNAs. Since then it has become apparent that the 45S RNA contains the sequences of both the 28S and 18S RNA molecules, which together occupy about 7,500 nucleotides of its total length of 14,000 bases.

In bacteria the situation at first appeared to be different, with the 16S and 23S rRNAs each apparently transcribed independently, although their genes were located adjacent on the chromosome. But the isolation of an *E.coli* strain lacking a processing enzyme allowed Nikolaev, Silengo and Schlessinger (*Proc. natn. Acad. Sci U S A.*, **70**, 3361–3365; 1973) and Dunn and Studier (*ibid.*, 3296–3300) to isolate a 30S precursor molecule that contains both sequences.

How are the mature rRNA sequences organised within the precursor? In bacteria the sequences 5′ 16S–23S–3′ is generally agreed on the basis of the relative sensitivity of 16S and 24S RNA synthesis to inhibition by rifampicin (for example Doolittle and Pace, *Proc. natn. Acad. Sci. U.S A.*, **68**, 1786–1790; 1971). In eukaryotes the evidence has been conflicting although up to now it was generally held to favour the reverse order. In particular the identification of distinct secondary structure of the various rRNAs and their precursors in the electron microscope and the determination of polarity by digestion with a 3′ exonuclease, suggested that the 5′ end of the 28S molecule coincides with the 5′ end of the 45S precursor (Wellauer and Dawid, *Proc. natn. Acad. Sci. U.S A.*, **70**, 2327–2831; 1973). This seemed to be the definitive answer and a similar organisation for *Xenopus* 40S precursor was suggested by the same authors (*J. molec. Biol.*, **89**, 379–396; 1974).

But two recent experiments using different techniques suggest the opposite conclusion. Hackett and Sauerbier (*J. molec Biol.*, **91**, 235–256; 1975) inhibited RNA synthesis in L cells by ultraviolet irradiation. The irradiation causes transcription to terminate at damaged sites in DNA; the farther a gene lies from its promotor, the more likely it becomes that a terminating event will occur before its transcription is completed The pseudo-first order loss of 45S RNA as a function of ultraviolet dose confirmed that each gene for the precursor represents an individual transcription unit with its own promotor. The target size of the 18S cistron suggested by these experiments was about 4,000 nucleotides, just twice the length of the 18S RNA molecule. This suggests that a spacer of 2,000 nucleotides lies at the 5′ end of the 45S precursor, followed by the 2,000 nucleotides of the 18S sequence. The target size of the 28S RNA was 11,000 nucleotides; since the molecule is 5,500 nucleotides long, it must begin at a point 15,000 nucleotides after the end of the 18S RNA This leaves a distance of 3,000 nucleotides at the 3′ end of the 45S RNA, which must be part of the discarded sequences. The target size of the 32S precursor, the immediate precursor of 28S RNA, is the same as that of the 28S molecule; this suggests that the 32S precursor is adjacent to the 18S sequence, so that the 45S RNA has the structure:

$$5'\ \underset{\text{Spacer}}{\longleftarrow\!\!-\!\!-}\times\underset{\text{18S}}{-\!\!-}\times\overset{\text{32S}}{\underset{\text{28S}}{\longleftrightarrow}}\underset{\text{Spacer}}{-\!\!-\!\!\longrightarrow}\ 3'$$

Another approach to analysing sequence organisation has been taken by Liau and Hurlbert (*J molec. Biol.*, **98**, 321–332; 1975). The mature 28S and 18S RNA molecules are methylated on the 45S precursor, when only these sequences gain the methyl groups. The relative amounts of methylation of the two mature molecules are different, so that methylation could be used to identify the parts of the 45S precursor carrying these sequences. The experimental problem is to obtain identified fragments of the 45S molecule. Liau and Hurlbert generated suitable conditions by incubating Novikoff rat tumour nucleoli in the absence of nucleotides; this prevents elongation of polynucleotide chains, generating a series of incomplete precursor molecules of varying lengths starting from the 5′ end, which are methylated *in vitro*. The 18S methylation pattern is found in the shorter transcriptions and the 28S pattern is seen in the longer molecules.

One of the most satisfying conclusions to be drawn is that the same relative organisation of mature rRNA sequences within the precursor is seen in bacterial and eukaryotic systems. The smaller molecule precedes the larger, although more extensive spacer sequences have evolved in the eukaryotic systems. It seems clear that the relative locations of the 18S and 28S RNA sequences seen in the HeLa and *Xenopus* precursors by electron microscopy are indeed correct; but the polarity is reversed. The explanation seems to lie in the use of the exonuclease to determine the polarity of the RNA; clearly in the system used, the 5′ rather than the 3′ end must have been digested, perhaps by some contaminating enzyme.

What is the function of the spacer regions in the common precursor? Little information is available on the extensive spacers of the eukaryotic precursor, but the striking observation that a tRNA gene lies between the 16S and 23S sequences in an *E.coli* unit has been reported by Lund *et al.* (*Cell*, **7**, 165–177; 1976). The *E.coli* genome possesses several copies of the genes for rRNA, organised in transcription units that display the sequence 16S–23S–5S RNA (with the assignment of the 5S RNA to the end of the unit being less certain than the tandem arrangement of the 16S and 23S sequences). The transducing phage $\lambda rif^{d}18$ carries one of the sets of the genes for 16S and 23S RNA, as well as genes for some RNA polymerase subunits, ribosomal proteins, and a protein synthesis factor. When cells are infected with this phage, the synthesis of two small RNA species is greatly stimulated: one is 5S RNA and the other is $\mathrm{tRNA}_2^{\mathrm{Glu}}$. Where is the tRNA gene located? By showing that a radioactively labelled $\mathrm{tRNA}_2^{\mathrm{Glu}}$ molecule could hybridise to a phage DNA deleted for the 23S sequence, but could not hybridise to a phage DNA where the deletion extended from the 23S sequence into the 16S sequence, they were able to conclude that the gene for the tRNA must lie between the genes for the 16S and 23S RNAs (Yamamoto, Lindahl and Nomura, *Cell*, **7**, 179–190; 1976). This location was confirmed by Wu and Davidson (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 4506–4510; 1975). in experiments utilising a new technique for visualising RNA–DNA duplex regions formed along a single strand of DNA.

An obvious question is whether this form of organisation is seen in all the 16S–23S transcription units of the bacterial genome. Lund *et al.* (*op. cit.*) examined the transducing phage $\phi 80 rif^{r}$, which also carries an rRNA transcription unit, and showed that it is homologous with the $\lambda rif^{d}18$ phage over most of the bacterial sequences except for 310 base pairs between the 16S and 23S rRNA sequences. This means that the internal spacers are different in the two transcription units. When cells are infected by $\phi 80 rif^{r}$ $\mathrm{tRNA}_1^{\mathrm{Ile}}$ is synthesised, and Lund *et al.* have confirmed that its gene seems to lie within the spacer region. At least two of the rRNA transcription units of *E.coli* therefore possess a (different) tRNA sequence; whether the other (approximately 5) units also carry tRNA sequences remains to be established. What function in bacterial protein synthesis might be served by this coupling is not yet clear, although it seems to have the consequence that the tRNA coded with the rRNAs is synthesised in large amounts (both $\mathrm{tRNA}_2^{\mathrm{Glu}}$ and $\mathrm{tRNA}_1^{\mathrm{Ile}}$ are among the

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The Red Queen lives

from Leigh Van Valen

When the Red Queen heard of her reported dethronement (Hallam, *Nature*, **259**, 12–13; 1976), she smiled from her throne and said, "As Mark Twain remarked on a report of his death, I find the report somewhat exaggerated".

One side of a controversy is often convincing until the other side appears. Hallam primarily based his conclusion on two recent papers of Salthe and Sepkoski; my response to these has not yet been published and will be part of a broader discussion For now I summarise my comments on the points Hallam discussed.

There are several possible hypotheses to explain the law of constant extinction, which produces a linearity of the survivorship curves of adaptively similar groups on a semilog plot. One is the Red Queen's Hypothesis, that the sum of absolute fitnesses in a community is constant. Another, used by Raup *et al.* (*J. Geol.*, **81**, [for 1973], 525–543; 1974) in their simulations and which I call the Field-of-Bullets Hypothesis, is that the probability of extinction is constant for all groups at all times. This hypothesis is unlikely (Van Valen, *Evol. Theory*, **1**, 1–30; 1973; *Nature*, **257**, 515–516; 1975). A third hypothesis, which Salthe (*Paleobiology*, **1**, 356–358; 1975) proposed and which may be called the Hypothesis of Triviality, is that any reasonable pattern of causation leads to near linearity and so no specific explanation is appropriate. There are several problems with the Hypothesis of Triviality, but it would seem to be disproved by the existence of a few systematically non-linear survivorship curves. That such deviations from linearity have known no possible particular explanations (Van Valen, *Evol. Theory*, **1**, 1–30; 1973) merely accentuates their reality. Simpson (*Tempo and Mode in Evolution*; 1944) used a phenomenon equivalent to part of this exceptional non-linearity as the basis for distinguishing what he called bradytely and horotely as more or less discrete evolutionary modes. Horotely is intimately related to linearity or survivorship curves.

Sepkoski (*Paleobiology*, **1**, 343–355; 1975) in fact claims that most of the curves are non-linear and that the distribution of the lengths of stratigraphic units produces a bias that gives spurious linearity. However, the main result of this bias, when I apply my methods to his simulations, is a reduction in the proportion of bradytelic taxa that are identified, rather than a qualitatively false conclusion of linearity and homogeneity. Sepkoski's analyses were mostly of situations more extreme than the ones I used and most of his results have little bearing on mine. I originally excluded some data from any analysis because of related inadequacies. There are many kinds of inaccuracies involved in my analysis, some as yet unpublished, but none known to me seriously affect the predominant linearity of the curves.

The Red Queen's Hypothesis is ecological and does not depend on the linearity of taxonomic survivorship curves which served as midwife. Her domain is less than universal and may be empty: the hypothetico-deductive method is weak, and the sort of facts from which the hypothesis may be deduced are as yet only suggestively available. But the Red Queen is rather flexible, as a runner should be, and her domain may yet include much of evolution.

more abundant of the *E.coli* tRNAs). The processing of the transcript of the complete unit releases four RNA species, in the order 16S, tRNA, 23S, 5S RNA; the enzyme that appears to undertake this reaction is ribonuclease III and by showing that a deletion phage lacking the end of the 16S sequence can still generate 23S and tRNA sequences, Yamamoto *et al.* (*op. cit.*) were able to infer that the complete precursor sequence is not required for proper processing. This supports the idea that processing can occur before transcription has been completed. ☐

Nuclear charge distributions

from P. E. Hodgson

NEW measurements of the cross section for the elastic scattering of electrons by lead have recently cleared up an outstanding discrepancy concerning the charge distribution in the interior of the nucleus. It has for a long time been uncertain whether there is a dip or a hump in the middle of ^{208}Pb, and it has now been established that there is a small hump.

At first sight this result seems surprising. We know that the protons in the nucleus repel each other by their electrostatic charges, and this might lead us to expect a dip in the charge distribution in the middle of the nucleus. We must however think of the protons as occupying shell model orbits, and the total charge distribution is found by adding the squared moduli of the wavefunctions of the protons in all the occupied orbits. Now the wavefunctions of the protons in S-orbits, with zero orbital angular momentum, have a finite value at the centre of the nucleus, whereas the wavefunctions of protons in all other orbits are zero at the centre. The value of the charge density at the centre of the nucleus thus depends quite critically on the number of protons in S-orbits. In lead the protons in the 3S orbits have the smallest binding energy, and so we might expect that these form a hump at the centre of the charge distribution, and this hump is indeed given by most calculations of the charge distribution of lead, for example those made using the Hartree–Fock theory.

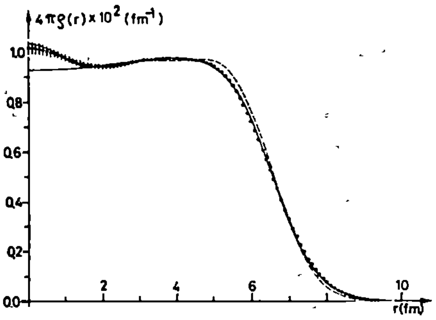
Until recently, however, the experimental determinations of the charge distribution have tended to give a small central dip, and this could not be reconciled with the theoretical result. There was however some uncertainty about this because of the difficulty of obtaining the charge distribution from the measurements of electron scattering.

Electrons only interact with the nucleus throughout their electromagnetic field, so if we scatter electrons by nuclei we should learn about the charge distribution of the nucleus. Unfortunately, although it is possible to calculate the scattering from a known charge distribution, it is not easy to reverse the calculation and obtain the charge distribution from the electron elastic scattering cross section Some quite sophisticated techniques have been developed to do this, and the differences between them account for much of the uncertainty about the form of the charge distribution in the centre of the nucleus.

The simplest method is to assume a suitable analytic form for the charge distribution and then vary its parameters to optimise the fit to the electron elastic scattering cross section. One of the most popular forms is the Fermi function $[1+\exp\{(r-R)/d\}]^{-1}$ which is uniform in the interior and falls rapidly to zero around the radial distance R, the steepness of fall depending on the parameter d. This was soon found to be insufficiently flexible, so it was multiplied by various polynomials such as $(1+wr^2)$, which makes it possible to introduce dips and humps in the interior.

Many analyses were made in this way, and it was often possible to obtain excellent fits to the scattering cross sections, but there was some doubt about the interpretation of the resulting charge distributions. It was realised that the scattering is most sensitive to the charge distribution in the 'knee' region at the surface of the nucleus, and the parameters are largely determined by the behaviour of the assumed charge distribution in this region, so that it is dangerous to assume that this gives us reliable information about the charge distribution in other regions such as the centre of the nucleus.

Much effort has therefore been devoted to the development of the so-called model-independent ways of determining the charge distribution from the scattering cross sections. Some of these use a series of concentric shells of charge, and adjust the densities of each, while others express the charge distribution as the sum of a series of terms each consisting of a suitable function of the radial distance, with different ranges and coefficients. Some important work of this type has been done by Friedrich and Lenz (*Nucl. Phys*, **A183**, 532; 1972) and Friar and Negele (*Nucl. Phys*, **A212**, 93; 1973), so that it is now possible to obtain the charge distribution without making any unduly restrictive assumptions about the form of the distribution.



The charge distribution of lead, as determined experimentally and by Hartree–Fock calculations. The experimental points correspond to the analysis of the Mainz data, the dashed line to the calculations of Vautherin and Brink using the Skyrme force, and the full line the result of an analysis using a three-parameter Fermi distribution that does not give an acceptable fit to the electron scattering data.

The new measurements that have recently been made to resolve the difficulty about the charge distribution in the centre of lead are due to the group of Professor Ehrenberg at the University of Mainz (Euteneuer, Friedrich and Voegler, *Phys. Rev. Lett.*, **36**, 129; 1976). They scattered electrons of 120, 200 and 290 MeV from lead, and analysed the results using a very flexible expansion of the charge distribution in terms of a series of spherical Bessel functions of various ranges. Their results, as shown in the figure, provide definite evidence in favour of a hump in the centre of the nucleus, in accord with the theoretical calculations.

This work shows how accurately we now know the charge distribution of some nuclei. There still remain some uncertainties, especially in the region of low densities at the edge of the nucleus, and this will doubtless be the subject of further studies using electrons of higher energies. ☐

Role for histone H1 in chromatin condensation?

from Tom Barrett

THE lysine-rich histone H1 is the most unusual of the five major histone species found in eukaryotic chromatin In contrast to the other histones which show a strict sequence conservation over a very long evolutionary period, the amino acid sequence of histone H1 varies to some extent (Rall and Cole, *J. biol. Chem.*, **246**, 7175; 1971). Its molecular weight (about 23,000 daltons) is greater than that of the other major histone species H2A, H2B, H3 and H4. It contains a very high proportion of basic amino acids; approximately 25% of the molecule is composed of lysine residues. It is the most readily removed of all the histone species in spite of its very basic charge and can be washed off chromatin with 0.5 M NaCl. Immunological studies have shown that its antigenic determinants are more available in native chromatin than are those of the other histone species. In percentage terms its immunological availability at 9.6% is considerably greater than that of the next most available histone—H2B at 3.2% (Goldblatt and Berstin, *Biochemistry,* **14,** 1689; 1975). Crosslinking experiments performed on chromatin show that histone H1 does not readily form complexes with other histones but rather forms H1 oligomers (Thomas and Kornberg, *FEBS Lett.,* **58,** 353; 1975).

These differences are highlighted by the fact that histone H1 is not required for formation of the characteristic bead-like structures, called ν bodies or nucleosomes, found in native eukaryotic chromatin. Nucleosomes consist of a histone core made up of two each of the four histones H2A, H2B, H3 and H4 associated with approximately 200 base pairs of DNA. Recent neutron diffraction studies indicate that the DNA is wound in a superhelix around the outside of this histone core (Baldwin *et al., Nature,* **253,** 245; 1975; Pardon *et al., Nucl. Acids Res.,* **2,** 2163; 1975).

Chromatin condensation

Since histone H1 is not involved in the formation of the basic nucleosome structure of chromatin its function must be independent of that of the other histone species. There is strong evidence that histone H1 is responsible for chromatin condensation. In dilute buffer solutions chromatin forms a viscous gel which contracts to a maximum of 10% of its original volume as the ionic strength is increased. Increasing the ionic strength above a certain level causes the chromatin to expand again as the histone H1 begins to dissociate from it. The release of H1 from chromatin is governed by the effective ionic strength of the cationic component of the salt, 0.5 to 0.6 M being the critical concentration for release of H1 (Bradbury *et al., Eur. J. Biochem;* **57,** 97; 1975). These authors have made an extensive nuclear magnetic resonance (NMR) study of histone H1 both in free solution and in chromatin under various ionic conditions. In ^2H_2O virtually all the H1 resonance spectrum can be seen but the signals are broader than those of isolated H1. Addition of salt causes the resonances to broaden until a concentration of 0.4 M salt is reached when the spectrum begins to sharpen. When 0.5 to 0.55 M is reached the H1 spectrum is fully developed with a marked increase in the intensity of the lysine resonances. Broadening of the peaks thus corresponds with the contraction of the chromatin gel. The contraction of the chromatin gel is not observed with H1-depleted chromatin. Spectra obtained for H1–DNA complexes are identical in nearly all respects with those obtained for chromatin under the same solvent conditions. The main difference is that the spectrum for the H1–DNA complex is not as broad as that of chromatin at low ionic strength and is evidence that there are more free H1 lysine residues in the complex than in chromatin. Probably one third of the lysine residues in the complex are free in these conditions in spite of the very high positive charge on the lysine molecule. The authors suggest that the addition of salt causes a structural change in H1 and/or reduces the repulsion between the DNA molecules, allowing them to approach each other more closely and bind the free end of the H1 molecule. In chromatin the other histone molecules are present and act to reduce the high charge on the DNA molecules.

Both the crosslinking experiments and the NMR resonance spectra indicate that in chromatin histone H1 is not associated with the other histones but is bound directly to the DNA. Structurally, histone H1 consists of an apolar-rich central region—residues 40 to 75—a short N-terminal end and a long C-terminal end rich in basic residues. The central apolar region is probably the site where the histone H1 molecules interact with one another. The long C-terminal end is the most basic region of the molecule and is probably the main site for the interaction of H1 with both free DNA and chromatin DNA (Bradbury *et al., Eur. J. Biochem;* **52,** 605; 1975). It has been reported recently that the aggregation of DNA molecules by histone H1 is much greater with superhelical DNA, irrespective of the sign of the superhelical turns, than with open relaxed DNA (Bottger *et al., Nucl. Acids Res.,* **3,** 419; 1976). Since the DNA in the nucleosome appears to be supercoiled around the histone core this structure would favour the interaction of histone H1 with the DNA.

Whether or not histone H1 is associated with the DNA wrapped around the nucleosome proper or to the DNA linking adjacent nucleosomes is not certain. A recent paper by Varshavsky *et al.* (*Nucl Acids Res.,* **3,** 477; 1976), however, lends support to the latter idea. They purified nucleosome monomers on sucrose gradients and on subsequent electrophoresis on polyacrylamide gels found two discrete types of nucleosome. The first contained all five histones associated with 200 base pairs of DNA, whereas the second lacked histone H1 and contained only 170 base pairs of DNA. They could also distinguish three types of dimer containing 0, 1 and 2 molecules of histone H1 respectively. They suggest that H1 is bound to a short (30 base pair) terminal stretch of nucleosomal DNA which can be removed by nuclease treatment, probably as a H1–DNA complex, without significantly disturbing the basic nucleosome structure.

Chromatin transcription

Histone H1 is absent or very much reduced in concentration in chromatin that is being actively transcribed. Calf thymus chromatin can be resolved into two fractions by chromatography on ETCHAM-cellulose. The first fraction to elute contains histone H1 whereas the second fraction is depleted in H1. This H1-depleted fraction is enriched in RNA polymerase binding sites and presumably corresponds to the active chromatin (Simpson, *Proc. natn. Acad. Sci., U.S.A.,* **71,** 2740; 1974). Using a different technique whereby Mg^{2+} is used to precipitate the condensed or inactive chromatin fraction, Gottesfeld *et al* (*Proc. natn. Acad. Sci. U.S.A.,* **71,** 2193; 1974) separated a soluble nucleoprotein fraction from rat liver chromatin. This fraction was enriched several-fold in transcribed DNA sequences and histone H1 was virtually absent. In the inactive, highly condensed chromatin of nucleated erythrocytes an additional histone species, histone H5 is present. In many respects H5 is similar to H1 and removal of both these histones from erythrocyte chromatin causes it to expand and resemble the uncondensed fraction of rat liver chromatin. Histone H1 seems to substitute equally well for H5 in the condensation of erythrocyte chromatin. It is probable that the highly condensed state of erythrocyte chromatin is not due solely to the presence of the erythrocyte-specific histone H5 but rather to a greater saturation of the H5 binding sites in erythrocyte chromatin. Reeck (*Arch. Biochem. Biophys.,* **172,** 117; 1976) could detect no H5-depleted regions analagous to the H1-depleted regions in calf thymus chromatin. In fact during the erythrocyte maturation process the concentration of H5 increases in parallel with a decrease in RNA synthesis (Billet and Hindley, *Eur. J. Biochem.,* **28,** 451; 1972).

There is good evidence that the other histone species are present in actively transcribed chromatin. The presence of nucleosomes on DNA does not seem to block its transcription. Lacy and Axel (*Proc. natn. Acad. Sci. U.S.A.,* **72,** 3978; 1975) isolated monomeric nucleosomes from rat liver chromatin and found that a further 46% of the DNA in the monomers could be digested. Analysis of the reassociation kinetics of the resistant DNA revealed that most, if not all, the genomic sequences were involved in this basic subunit structure and that virtually all the liver sequences transcribed into nuclear RNA were present in the nucleosomal DNA.

It appears that the histones act in two distinct ways to fold the DNA molecule in chromatin. First, the four histones H2A, H2B, H3, H4 form an octameric protein core around which the DNA is supercoiled. This results in a 7-fold contraction of the DNA but does not block transcription. Second, histone H1, when present, further condenses the nucleohistone and in so doing makes it unavailable for transcription. ☐

Active Mercurian dynamo unnecessary

from Peter J. Smith

According to measurements made recently by Mariner 10, the planet Mercury has a magnetic dipole moment of about 5×10^{22} gauss cm^3 (5×10^{18} A m^2), which is more than three orders of magnitude smaller than that of the Earth and over 10,000 times larger than the upper limit of the lunar dipole. But what is the origin of Mercury's magnetism? The maximum field observed in the vicinity of the planet by Mariner 10 was 400 nT which, being 20 times greater than the interplanetary field, led Ness *et al.* (*Nature*, **255**, 204; 1975) to rule out induction processes as a source. It seems more likely, therefore, that the field is of internal origin.

And of the various theoretically possible internal sources, Ness and his colleagues favoured a currently active dynamo in what would have to be a very large iron–nickel core. They tentatively rejected remanent magnetisation of parts of the planet on the grounds that it is "difficult to construct a plausible sequence of events leading to the model in which the Mercurian magnetic field is explained in [those] terms". Moreover, shortly before this, Runcorn (*Nature*, **253**, 701; 1975) had shown that the external dipole moment of a shell magnetised in the field of an internal dipole which has since disappeared is apparently zero.

But according to Stephenson (*Earth planet. Sci. Lett*, **28**, 454; 1976), remanent magnetism as the source of Mercury's dipole moment cannot be rejected so easily. His new analysis indicates that an outer magnetised shell will give rise to a residual dipole moment if there is a difference in permeability between the shell and the planetary interior or between the shell and free space. Assuming the Mercurian surface rocks to be similar to those of the Moon, it may then be shown that a magnetic shell could quite easily account for the observed dipole moment without invoking ancient magnetising fields of more than a few gauss, an iron content of more than a few percent, or a shell thickness of more than a few tens of kilometres.

In short, contrary to previous belief, it is not necessary to conclude from the magnetic field observations that Mercury still has an active dynamo. The internal source options remain open.

The illogic of conservation

from Peter D. Moore

It is difficult to be objective, or even rational, about conservation. This is especially true in situations where the conservationist is concerned with the continued survival of a species whose population has fallen to a critically low level. When the very existence of a species is threatened then the conservationist does have sound reason on his side, for extinction, and the depletion of the world's genetic resources which this represents, cannot be regarded dispassionately, yet in practice the attitudes of those who seek to guard the world's wildlife are often far more parochial and far less guided by logic than is normally supposed.

It is this criticism which John Gooders (*Brit Birds*, **69**, 16; 1976) has recently levelled at ornithologists. He believes that a disproportionate amount of concern and money is spent upon preserving isolated colonies of bird species in the peripheral areas of their global range simply because they are rare there and because the birdwatcher in the street has a consuming interest and is therefore willing to spend money on propping up rarities, even if they are common elsewhere in the world. Certainly it is not difficult to find examples of this kind of behaviour on the part of British birdwatchers. Gooders quotes such birds as the snowy owl (*Nyctea scandiaca*), osprey (*Pandion haliaetus*) and avocet (*Recurvirostra avosetta*) all of which are widespread globally and are far from being a threatened species and yet which have received an inordinate amount of attention from British ornithologists because they have recently been able to invade, or reinvade, these islands as breeding birds. Would we not do better, Gooders argues, to concentrate on our ornithological resources which are truly of international importance, particularly in the conservation of such habitats as our estuaries which are vital for many species of wildfowl and wader.

One must sympathise with Gooders' view, and it is true that the British are not alone in this parochial attitude, nor is it confined to the ornithologists. In Estonia biologists pride themselves on having the mute swan (*Cygnus olor*) as a breeding species and avidly protect the single Soviet site where the cross-leaved heath (*Erica tetralix*) is found growing (the species is abundant in more oceanic areas of western Europe). The Norwegians take delight in the gorse effort in trying to rid himself of the same species. The British botanist (*Ulex europaeus*) which just maintains itself in the south-western parts of their country, whilst the British hill farmer spends much time and fervently defends the few British plants of the military orchid (*Orchis militaris*) yet the plant is relatively frequent in parts of central Europe.

Indeed, the botanist is probably more regionally orientated than the ornithologist; the old county boundary is often his horizon. The Kentish man is proud of bog asphodel (*Narthecium ossifragum*) because it is rare in the south east of Britain, though common in the west, and in what was Warwickshire the crowberry (*Empetrum nigrum*) is prized, having only one station in the county, yet it is common further north

But we can really expect more of mortal, irrational man? Can one really expect people to be concerned about species which may be threatened on a global scale and yet, for them, will never be more than a picture in a wildlife magazine? For the panda, or the tiger it may be possible, but for the bald ibis unlikely. The average subscriber to conservation bodies and fund-raising organisations cannot think in international terms because his main concerns are with the local situation. His reasons for desiring conservation are not coldly scientific but are aesthetic and emotive. ☐

articles

Radiocarbon chronology for early Maya occupation at Cuello, Belize

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Radiocarbon dates from excavations at the prehistoric Maya site of Cuello, Belize, indicate occupation around 2600 BC *in calendar years, antedating the hitherto earliest known occupation of the Yucatan Peninsula by some 1,700 yr and demonstrating that sedentary pottery-using communities in the Maya lowlands may be as early as any in Mesoamerica.*

WE report here a series of radiocarbon determinations which place the origins of Maya settlement and civilisation in the Yucatan Peninsula back in the third millennium BC, some 1,700 yr earlier than the first occupation known until now[1]. Dating of the early phases of occupation of the Maya site of Cuello indicates a sedentary pottery-using settlement there by 2,000 yr b.c., in radiocarbon years, 2600 BC on the basis of the bristlecone pine calibration of radiocarbon chronology[2].

Background

Maya civilisation flourished in the Yucatan Peninsula and the adjacent highlands and Pacific slope through most of the first millennium AD, the Classic Period of greatest florescence being defined by the existence of dated inscribed monuments at about AD 250–900. Before this was a Preclassic or Formative Period going back to the earliest sedentary settlement of the Maya area and Mesoamerica generally, and formally defined as beginning in 2000 BC when it succeeded the long Archaic Period[3].

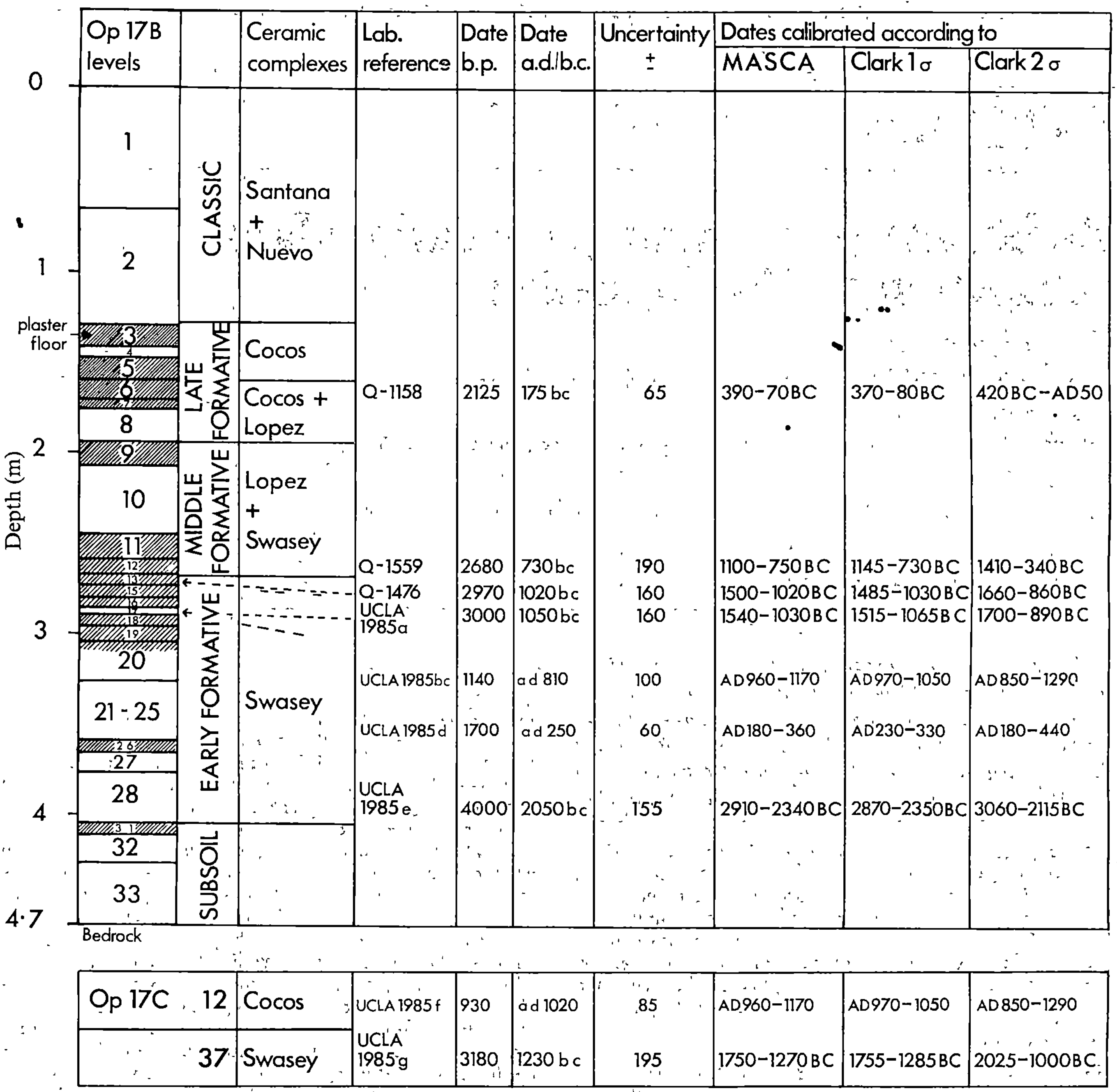
During the Formative Period, settled village agriculture became the major means of subsistence in Mesoamerica, with the use of pottery beginning between 2,500 and 2,000 yr b.c., in highland and Pacific Mexico. On this base, complex societies arose towards the end of the second millennium BC, and the Olmec civilisation emerged in the Gulf Coast lowlands of Mexico after 1,200 yr b.c. The earliest evidence for settlement in the Yucatan Peninsula and Usumacinta basin to the east is based on a single radiocarbon date of 660 yr b.c., 850 BC on the bristlecone pine calibration (UCLA-1,437; 5382b 2,610±75 yr b.p.) from the earliest Real Xe phase of the occupation of the major site of Seibal, Guatemala[1], which had Olmec associations. The determinations listed below demonstrate the contemporaneity of occupation of the Maya lowlands with the Olmec to the west, and, in the priority of the earliest date, provide another possible source for Olmec culture.

Cuello

The Cuello site, located in northern Belize at approximately 18°05′N, 88°35′W, is one of a number of Maya ceremonial centres excavated over the period 1973–75 by the Corozal Project, a joint venture of the British Museum and Cambridge University. Excavations in 1975 (supervised by Pring) revealed a deep stratigraphic sequence in Operation 17B of plaster platforms and midden deposits with bedrock at ~4.70 m below ground level; the middens contained partially burnt wood which has been used for the bulk of the radiocarbon determinations reported here, although one date (Q-1,476) is directly associated with a burial. The determinations were carried out at the University of California (UCLA) by Berger and at Cambridge University by Switsur and Ward. The radiocarbon determinations were all carried out using gas proportional detectors filled with purified carbon dioxide produced by the oxidation of the specimens of partially burnt wood from the excavations. The samples were all rather small, and where they yielded insufficient gas for filling the counters, additional radioactively inert carbon dioxide was added. Each sample contained traces of plaster, and this was eliminated by treatment with dilute mineral acid. The calculated radiocarbon dates quoted are based on the 'Libby' half life for radiocarbon of 5,568±30 yr.

All of the samples relate archaeologically to the Formative Period, before the institution of the practice of erecting dated stone monuments, and thus no equivalent dates in the Maya calendar are quoted, although for purposes of articulating the Christian and Maya calendars, we accept the Goodman–Martinez–Thompson correlation.[3] Dates in calendar years BC are quoted here on the basis of the MASCA calibration and that recently advanced by Clark[4], and together with the radiocarbon determination, excavation level and associated ceramic complex are displayed in Fig. 1. Determinations for the upper part of the stratigraphic sequence (levels 1–14) were made at Cambridge, those for the lower part (levels 15–28) at UCLA; the two series are mutually consistent.

The uppermost levels (1–2) of the excavation Operation 17B contained material of the Early and Late Classic Periods (AD 250–900), overlying a plaster floor (3) which covered a sequence of Formative Period deposits[5]. Levels 3–5 contained pure Late Formative ceramics of the Cocos Chicanel ceramic complex and levels 6–8 Cocos material mixed with ceramics of the



Op 17B levels	Ceramic complexes	Lab. reference	Date b.p.	Date a.d./b.c.	Uncertainty ±	MASCA	Clark 1σ	Clark 2σ
6–7	Cocos + Lopez	Q-1158	2125	175 bc	65	390-70BC	370-80BC	420BC-AD50
12	Lopez + Swasey	Q-1559	2680	730bc	190	1100-750BC	1145-730BC	1410-340BC
14		Q-1476	2970	1020bc	160	1500-1020BC	1485-1030BC	1660-860BC
17		UCLA 1985a	3000	1050bc	160	1540-1030BC	1515-1065BC	1700-890BC
20	Swasey	UCLA 1985bc	1140	ad 810	100	AD960-1170	AD970-1050	AD850-1290
21-25	Swasey	UCLA 1985d	1700	ad 250	60	AD180-360	AD230-330	AD180-440
28	Swasey	UCLA 1985e	4000	2050bc	155	2910-2340BC	2870-2350BC	3060-2115BC
Op 17C 12	Cocos	UCLA 1985f	930	ad 1020	85	AD960-1170	AD970-1050	AD850-1290
Op 17C 37	Swasey	UCLA 1985g	3180	1230 b c	195	1750-1270BC	1755-1285BC	2025-1000BC

Fig. 1 Radiocarbon dates from Cuello, Belize. The dates are ranged against a scaled stratigraphic diagram (left) and major period and ceramic complex designations. Dates considered archaeologically unacceptable are printed in smaller type.

preceding Lopez Mamom complex. A date for a sample of burnt wood from level 6 of 2,125±100 yr b.p., 175 yr b.c. (Q-1558) falls well within the accepted span of 300 BC–AD 250 for the Late Formative Chicanel sphere, and the calibrated date is close to the radiocarbon result.

Levels 9–12 contained Middle Formative Lopez ceramics mixed with those of the Early Formative Swasey complex. The result of ^{14}C determination for a small sample of burnt wood (which had to be diluted with inert CO_2 for counting) from level 12 yielded a date of 2,680±200 yr b.p., 730 yr b.c. (Q-1559), calibrating to ~910 BC. The formal lower limit of the Middle Formative has previously been interpreted as ~800 BC, but the Real Xe material from Seibal also has a calibrated radiocarbon date beyond this limit (of ~850 BC); consequently on the basis of the chronometric evidence we propose that the limit should now be taken as at least 1000 BC.

Apart from a pit (level 21) penetrating down through levels 13–26 and containing Lopez complex ceramics, and a burial (level 14) cut into the plaster platform level 13, all subsequent stratigraphic levels in the excavation contained ceramics of only the Early Formative Swasey complex, the first indisputably Early Formative material to be found in the Maya Lowlands.

Level 14, a grave containing a single burial with three pottery vessels and a block of flint in place of a skull, yielded a sample of burnt wood from which a date of 2,970±160 yr b.p., 1,020 yr b.c. (Q-1476) was obtained, calibrating to ~1275 BC. Levels 13, 15 and 16 were three superimposed plaster surfaces, below which, level 17, of occupation debris, yielded a further sample of burnt wood giving a date of 3,000±160 yr b.p., 1,050 yr b.c. (UCLA-1985a), calibrating to 1320 BC; the Cambridge and UCLA dates for these two closely spaced levels are mutually consistent.

Levels 20 and 22 each yielded a very small burnt wood sample, which were amalgamated to give a date of 1,140±100 yr b.p., a.d. 810 yr (UCLA-1985bc), calibrating to AD 820. This date lies in the Late Classic Period, and is not archaeologically acceptable in view of the association with Early Formative ceramics. The level immediately below, 23, yielded a burnt wood sample which gave a date of 1,700±60 yr b.p., a.d. 250 yr (UCLA-1985d), calibrating to AD 280. This date falls at the end of the Late Formative, and is again archaeologically unacceptable on the basis of the associated ceramics. Both UCLA-1985bc and UCLA-1985d are also out of sequence with the later dates Q-1558, Q-1559, Q-1476 and UCLA-1985a, and the earlier date UCLA-1985e, which are consistent with each other, the stratigraphic sequence and the associated ceramics. We must therefore set aside these dates, and suggest that later disturbance, natural or anthropogenic, brought xylic material into these early levels; excavation of a larger area of the site in 1976 may provide evidence of this postulated disturbance.

Level 28 was a primary midden containing large quantities of Swasey Complex pottery, mollusc shells, animal bones and carbon flecks. It was sealed by the unbroken superimposed plaster surfaces levels 26 and 27, and partly overlay a rough limestone surface (level 31), which together with the rest of the midden deposit rested on the old land surface, level 32. The midden was 25–30 cm thick over the whole area of the excavation and represents the debris from a substantial and undoubtedly sedentary occupation of the site. A sample of burnt wood gave a determination of 4,000±155 yr b.p., 2,050 yr b.c. (UCLA-1985e), calibrating to ~2600 BC.

In another excavation at the site, Operation 17C, a major building recently half-destroyed by bulldozing for road metal was found to date to the terminal phase of the Late Formative, ~AD 200–250, on ceramic evidence. A construction level (12) yielded a sample of burnt wood which gave a determination of 930±85 yr b.p., a.d. 1,030 yr (UCLA-1985f), calibrating to AD 1,050. This date is archaeologically unacceptable, and could have resulted from either root penetration when the site was abandoned after the Late Classic collapse of Maya civilisation[6], or from an archaeologically undetected phase of Postclassic activity at the site.

Below the structure, in a level (37) of occupation material overlying limestone bedrock, a sample of burnt wood was recovered which gave a determination of 3,180±195 yr b.p., 1,230 yr b.c. (UCLA-1985g), calibrating to 1520 BC. This gives an approximate date to a burial sealed by level 37 and lying in a pit cut into bedrock, which was accompanied by five pottery vessels[5].

Discussion

The six acceptable determinations from the Cuello site together document a Formative sequence which, at its latter end, is compatible with what is already known about Maya Lowland and Mesoamerican chronology, but which, at its beginning, takes the prehistoric occupation of the Yucatan Peninsula back well into the third millenium BC. Although no other site in northern Belize has so far yielded similarly early radiocarbon dates, ceramics of the Swasey complex, which we must date to roughly 2,000–1,000 yr b.c. or 2600–1250 BC, have been recovered in Corozal Project excavations from three other sites, Nohmul, San Estevan and Santa Rita, mixed with later material but clear evidence of occupation at this early date. The existence of intensive agriculture utilising man-made raised fields along river and swamp margins a few km to the west by the end of the Swasey phase, is indicated by a radiocarbon date of 1,110±230 yr b.c. (I-7877A) for a trimmed post associated with such fields, calibrating to ~1400 BC, and maize pollen from a core taken at Laguna de Cocos may date to ~1800 BC[7]. In addition a possible early lithic site has been reported at Richmond Hill (D. Puleston, unpublished) only 3–4 km from the Cuello site, and although it remains undated at present an association with the Swasey phase inhabitants of Cuello is possible.

Data from pollen cores taken from Laguna de Petenxil in El Peten, Guatemala, 150 km south-west of the Cuello site, have suggested an agricultural occupation of that region ~2,000 yr b.c. on the basis of radiocarbon determination Y-1285 of 3,990±160, 2,040 yr b.c., calibrating to ~2590 BC[8], and this, has been extrapolated on the basis of sedimentation rates to suggest possible human interference with the vegetation of Peten ~4,000 yr b.c. (F. Wiseman, unpublished). This last proposal is still speculative; the Petenxil determination is, however, consistent with those from Cuello, and sedentary agricultural occupation of several areas of the rainforest zone by 2500 BC now seems likely. Such a date would accord with the still contentious glottochronological date for the splitting off of the Mayan language group.

On a broader Mesoamerican basis the radiocarbon chronology for Cuello may be compared with those for the Olmec area and the Central Mexican highlands. The latter part of the Swasey phase as defined by UCLA-1986g, UCLA-1985a and Q-1476 overlaps within 1σ the Bajio, Chicharras and San Lorenzo phases of the major early Olmec site of San Lorenzo Tenochtitlan[9]: the range for Cuello is 1,420–860 yr b.c., for San Lorenzo, 1,430–780 yr b.c. on 14 determinations. This range also overlaps the Tlatilco 'Olmec' and the Ayotla, Bomba and Justo phases in the Valley of Mexico, where the 1σ range of 5 determinations is 1,350–850 yr b.c. (ref. 10).

In the Maya highland zone the site of Chalchuapa in El Salvador has yielded two widely spaced dates for the Early Formative Tok complex of 2,790±60 yr b.p., 840 yr b.c. (P-1551), calibrating to 1020 BC and 3,610±60 yr b.p., 1,660 yr b.c. (P-1807), calibrating to ~2050 BC[11]. The later of these overlaps the latter end of the Cuello triad at the 1σ level, whilst the earlier lies between and overlaps UCLA-1985e and UCLA-1985g on 2σ.

The earliest ceramics so far reported in Mesoamerica are from the Puerto Marquez site on the Guerrero coast[12] where a determination of 4,390±140 yr b.p., 2,440 yr b.c. (Humble Oil Co; no laboratory number quoted), calibrating to ~3160 BC was obtained for a level containing mainly unslipped pottery termed 'Pox pottery'. Similar pottery was found in the Purron phase in the Tehuacan Valley in highland Mexico and placed by interpolation of dates at ~2,300 yr b.c. (2970 BC)[13].

Earlier ceramics are known from north-western South America, from Colombia and Ecuador[14], and from Panama at 4,090 yr b.p., 2,140±75 yr b.c. (Y-585), 2750 BC[15]. This pottery includes sophisticated slipped wares, as does the Swasey complex, while the Pox and Purron ceramics are mainly unslipped; the sample of Swasey material currently available is too small for dogmatic pronouncements on its resemblance and possible relationship to either the South American or the Mexican pottery to be made. Since the dates for Pox, Purron and Swasey all overlap at the 2σ level, it is an open question as to whether the earliest ceramics in Mesoamerica come from the Pacific or Atlantic coasts, or from the intervening highlands, and whether they are autochthonous or of ultimately, and perhaps not distantly, South American inspiration.

We thank the Trustees of the British Museum, the Research Committee of the British Academy and the Leverhulme Trust Fund for funding the project, which was based at the Centre of Latin American Studies, Cambridge, and directed by N.H.

Received February 9; accepted February 25, 1976

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Genetic hypertension in rats is accompanied by a defect in renal prostaglandin catabolism

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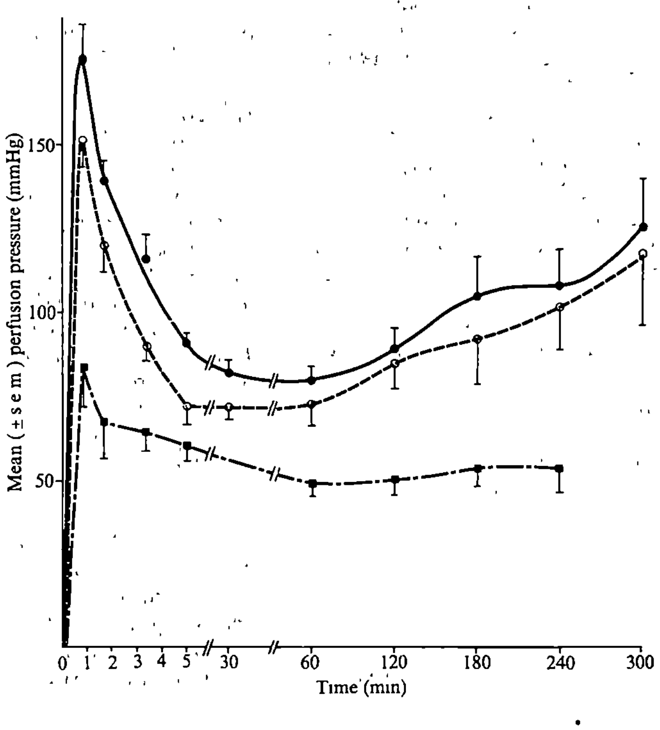
Noradrenaline releases prostaglandins in the kidney, and in rats these augment rather than reduce vasoconstriction produced by the amine. Homogenates of kidneys of New Zealand rats inbred for hypertension exhibit lower prostaglandin inactivation by 15-hydroxydehydrogenase than controls. At the same time, augmentation of noradrenaline vasoconstriction by the released prostaglandin is exaggerated. This biochemical defect could be the inherited abnormality primarily responsible for the development of hypertension in these animals.

In 1958 Smirk and Hall[1] reported that hypertension can be inherited in rats by selectively inbreeding animals with above average blood pressures. The strain which they developed, the New Zealand, genetic hypertensive (GH) rat has been studied extensively and similarities looked for between this model of the disease and essential hypertension in man[2]. The cause of hypertension is unknown in either species. Malik and McGiff[3] found that, in contrast to other species, prostaglandins of the E series cause vasoconstriction in the kidneys of rats. They also augment adrenergic transmission and potentiate the vasoconstrictor effects of noradrenaline. These results suggest that renal prostaglandins could contribute to the development of hypertension in the rat, as an increase in renal vascular resistance might be an initiating factor in the pathogenesis of the disease[4]. They also question the use of hypertensive rats as a model of human essential hypertension. Prostaglandin E_2 (PGE_2) is the principal renal prostaglandin (PG)[5,6] and is released from the kidney by pressor hormones[7,8]. We have, therefore, studied interactions of noradrenaline and PGE_2 in the renal vascular bed of normotensive and GH rats. Metabolism of PGs by kidneys from the two types of rat was also investigated to determine whether abnormal PG synthesis or degradation could contribute to the increased renal vascular reactivity which occurs in GH rats[9].

Male GH rats of the New Zealand strain of 17–19 weeks of age were used; their mean body weights (±s.e.m.) were 242.1±6.5 g and kidney weights were 1.56±0.04 g per pair. We used as control rats a Charles River strain which, like GH rats, originated from the Wistar strain. As the GH rats weighed less than normotensive control rats of the same age, kidneys were used from controls of either similar age (12 weeks) or body weight to those of GH. Systolic blood pressures, measured indirectly from the tails of rats anaesthetised lightly with ether before removal of the kidneys, were 160–190 mm Hg in GH rats and 110–130 mm Hg in normotensive rats.

Animals were anaesthetised with chloroform and the lower alimentary tract was excised after tying and severing the superior mesenteric, coeliac, spermatic and suprarenal arteries. Following intravenous heparin (100 IU per 100 g) and ligation of the aorta just below the coeliac artery stump, the kidneys were flushed with 5 ml oxygenated Krebs' solution by way of a cannula inserted into the abdominal aorta. The kidneys, attached to the cannula by the renal arteries and aorta, were rapidly transferred to the perfusion apparatus where they were hung from the aortic cannula in a covered water-jacketed glass container and perfused with oxygenated (95% O_2, 5% CO_2) Krebs' solution at 37 °C by a Watson–Marlow roller pump delivering 20 ml min^{-1}. This rate of perfusion flow, although greater than estimated by us to occur *in vivo*, has been found to prolong the useful life of similar preparations[10]. Furthermore, basal perfusion pressures were found to approximate more closely to normotensive blood pressures for rats *in vivo* (our unpublished observations). Perfusion pressure was measured by means of a Bell and Howell transducer placed proximal to the kidneys. The renal effluent was continuously assayed for PG-like activity by allowing it to superfuse in series a rat stomach strip, chick rectum and rat colon using the method of Ferreira and Vane[11]. Specificity and sensitivity of the assay tissues for PGs were increased by adding a mixture of pharmacological antagonists[12] and indomethacin (1–2 μg ml^{-1}) into the renal effluent before it superfused the tissues. Changes in length of the assay tissues were detected using Harvard transducers (type 386) and transcribed by a

Fig. 1 Graphs of mean pressures developed in the system at intervals after starting the perfusion of hypertensive or normotensive rat kidneys *in vitro*. Normotensives of body weight similar to those of GH were chosen as controls as their perfusion pressures were also similar. ——, Genetic hypertensive (n = 19); – – – –, Weight-matched normotensive (n = 12); – · – · –, Age-matched normotensive (n = 6). The number of preparations used in each group is given in parentheses.



Rikadenki KA-41 pen recorder. Drugs dissolved in saline were administered into a port situated proximal to the roller pump. Indomethacin was dissolved initially in 1 ml Tris buffer (*p*H 8) and the final concentration achieved by dilution with distilled water.

Selection of controls

Figure 1 compares changes, with time, of basal perfusion pressure of isolated kidneys of GH rats with those obtained from normotensive rats of either similar age or weight. The age-matched control group had a lower renal perfusion pressure, both at the start and at 5 h, than either the GH group or the weight-matched group. This difference could be accounted for by the differences in renal mass when identical rates of renal perfusion (20 ml min^{-1}) were used for the three groups. The kidneys obtained from GH and normotensive rats matched for body weight were comparable (1.6 g per pair), whereas those from age-matched normotensive rats weighed 2.3 g per pair, an increase in renal mass of about 40%. The heavier control kidneys had perfusion pressures which were 30–50% less than those of the other groups. Control rats were used with perfusion pressures similar to those of GH rats thus reducing the possibility that differences in vascular responsiveness between the two groups were a result of differences in basal tone of the vessels.

Release of PGs

Figure 2 is from an experiment in which different doses of noradrenaline were injected into the renal inflow. Dose-dependent increases in perfusion pressure were followed by the release of a substance which contracted the chick rectum and rat stomach strip superfused by the renal effluent. There was no effect on the rat colon. This pattern of activity could be matched by PGE$_2$ applied directly to the assay tissues. That PGs released from kidneys by noradrenaline were primarily of the E type, has been reported previously[3,13]. When indomethacin (1 μg ml^{-1}) was infused into the kidneys, the rises in perfusion pressure induced by noradrenaline were substantially reduced and there was no release of PGE-like activity[14].

Infusion of indomethacin into the kidneys of GH or control rats, although ensuring that its concentration in the superfusion fluid remained unaltered, was followed by relaxation of the assay tissues. This indicated that a resting output of PGE-like material had been abolished[15]. The level of basal release from the kidneys was estimated to be 0.1–1 ng ml^{-1} as these concentrations of PGE$_2$ were required to restore the tone of the assay tissues.

When PGE$_2$ was infused into the indomethacin-treated kidney, in amounts (100–300 pg ml^{-1}) which by themselves did not affect basal perfusion pressure, the pressor effects of noradrenaline were increased (Fig. 3*a*). This enhancement was sufficient to restore the effects of noradrenaline to pre-indomethacin levels. The potentiating effect of PGE$_2$ developed within 16–20 min and usually waned over a similar period after stopping the infusion The results of these experiments are summarised in Fig. 3*b*, *c* where changes in renal perfusion pressure produced by noradrenaline in GH and normotensive rats are shown, before and after inhibition of PG synthesis by indomethacin[14], the latter in the presence and absence of an infusion of PGE$_2$

Indomethacin usually did not affect basal renal perfusion pressure in normotensive rats, whereas in GH rats addition of indomethacin to the perfusing medium resulted in small reductions of 5–8 mmHg in perfusion pressure in 60% of these experiments. The failure of indomethacin to produce greater effects on renal perfusion pressure is consistent with the relatively small basal PG efflux from the rat kidney. Such small amounts would have marginal effects on basal tone but might still potentiate the renal vasoconstrictor activity of noradrenaline.

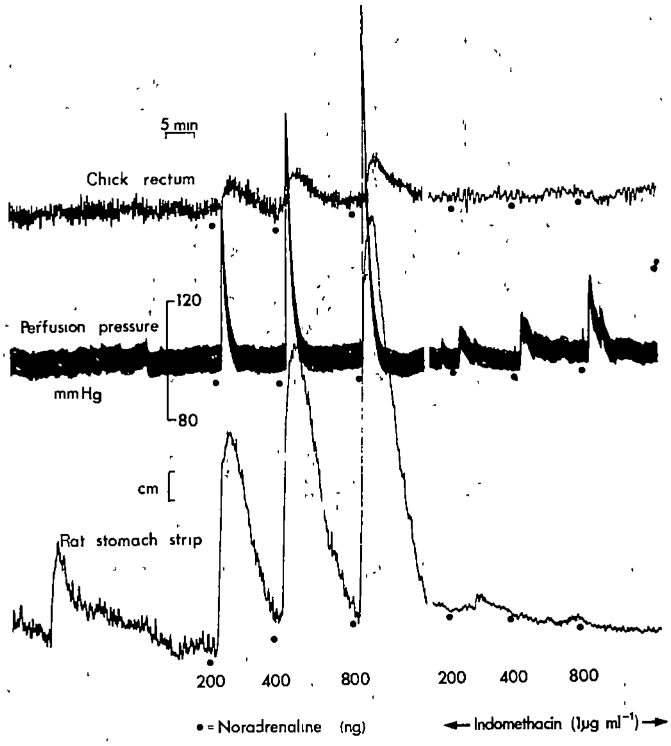


Fig. 2 The kidneys of a normotensive rat were perfused with Krebs' solution *in vitro* and the effluent allowed to superfuse assay tissues as described in the text. Indomethacin added to the perfusion fluid abolished the release of PGE-like activity, denoted by contractions of the chick rectum (top trace) and rat stomach strip (bottom trace), which normally accompanies the pressor responses (middle trace) evoked by noradrenaline. Moreover the rises in perfusion pressure produced by the amine were also markedly reduced in the presence of indomethacin.

In all these experiments we used the superfusion bioassay method to detect release of PGs by noradrenaline, to ascertain the effectiveness of indomethacin treatment in preventing release, and to determine the smallest dose of indomethacin which would achieve this effect. Since indomethacin inhibits PG dehydrogenase[16] and phosphodiesterase[17] in concentrations higher than those which affect synthetase, effects which by themselves could influence vascular reactivity, it was essential to select the minimal effective dose of indomethacin which inhibited prostaglandin synthetase. Our unpublished observations indicate that the concentration of indomethacin used (1–2 μg ml^{-1}) in this study does not substantially inhibit PG 15-hydroxydehydrogenase. These findings confirm those of Pace-Asciak[16].

Comparison of the vasoconstrictor responses to noradrenaline administered by injection in random sequence revealed that the renal vasculature of GH rats, compared with that of normotensive rats, was about 50% more sensitive to noradrenaline and was more susceptible to indomethacin-induced attenuation of the constrictor action of noradrenaline (Fig. 3*b*, *c*). In normotensive rats, PGE$_2$ (100–300 pg ml^{-1}) restored to pre-indomethacin levels the depressed renal vasoconstriction to doses of noradrenaline of 200 ng or greater, but not to low doses (50–100 ng). In contrast, similar concentrations of PGE$_2$ restored renal vascular reactivity of GH rats to low doses of noradrenaline, whereas restoration of responses to pre-indomethacin levels was incomplete to doses of the amine greater than 200 ng. The latter was related in part to the greater reduction in renal vascular responsiveness to noradrenaline produced by indomethacin in GH rats. Thus, the magnitude of PGE$_2$-induced potentiation of doses of noradrenaline greater than 100 ng in the presence of indomethacin was similar in both types of kidneys. The failure of exogenous PGE$_2$ to restore

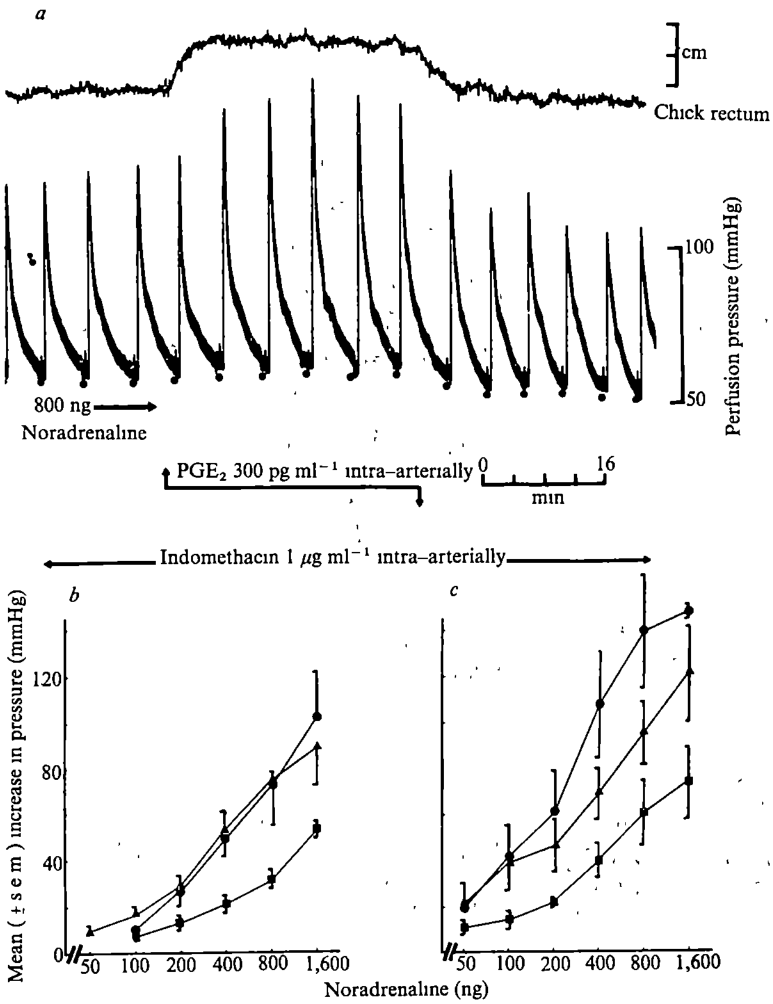


Fig. 3 *a*, Changes in perfusion pressure in response to intra-arterial noradrenaline (800 ng) injected at the dots, into the kidneys of a normotensive rat perfused *in vitro* with Krebs' solution containing indomethacin (for method see text). PGE_2, in amounts subthreshold for vasoconstriction, potentiated the vasoconstrictor responses to noradrenaline. The potentiation declined after withdrawal of the PG. *b*, *c*, Dose–response curves relating the vasoconstrictor responses to various doses of noradrenaline injected into the kidneys of normotensive (*b*, n = 7–10) or hypertensive (*c*, n = 7) rats *in vitro*. Addition of indomethacin to the perfusion fluid reduced in either group responses to each dose of the amine used. Thereafter infusion of PGE_2, in amounts subthreshold for vasoconstriction, augmented responses in either group. ●, Initial; ▲, indomethacin + PGE_2 (100–300 pg ml^{-1}); ■, indomethacin (1–2 μg ml^{-1}).

vasoconstrictor responses to noradrenaline may perhaps be a consequence of a reduced protein synthesis *in vitro* which would lead to lowered dehydrogenase concentrations[18] and thereby less potentiation than one would otherwise expect.

Of the other PGs identified in the rat kidney[5], $PGF_{2\alpha}$ in concentrations up to 10 ng ml^{-1} did not affect the renal vascular responsiveness to noradrenaline, whereas PGD_2 resembled PGE_2 in its ability to potentiate the amine.

The finding that kidneys of normotensive rats were less sensitive to inhibition by indomethacin of the vasoconstrictor action of noradrenaline could indicate that a smaller contribution was made by released PGs to the vascular effects of the amine than was the case in GH kidneys. Indomethacin caused a larger absolute reduction in vascular reactivity to noradrenaline in kidneys of GH rats than in those of normotensive rats, the renal vasoconstrictor responsiveness to noradrenaline was least in normotensive rats after indomethacin treatment. The difference which was revealed by indomethacin between the kidneys of GH and normotensive rats in their vascular responses to noradrenaline, that is, when the contribution of PGs was eliminated, is important since it indicates that other factors, such as structural changes in the vascular wall[19], also contribute to the increased vascular sensitivity to noradrenaline in GH rats.

Renal PG metabolism

Our results suggest that increased renal vascular reactivity to pressor hormones in the GH rat[20], could result partly from elevated levels of PGs Indeed, the renal vascular responsiveness to noradrenaline was reduced by indomethacin to a greater extent in the GH rat than in the normotensive rat (Fig. 3*b*, *c*). If increased levels of PGs are present, this could result from either increased synthesis or decreased degradation. Production of PGs by homogenates of kidneys of GH rats was, however, not different from normal as indicated by measurements of conversion of labelled arachidonic acid to PGs (Table 1). Moreover, no change could be detected in the pattern of production of PGs; that is, the rate of synthesis of PGE_2 was similar to that of $PGF_{2\alpha}$ in normotensive and GH rats; (PGE–PGF) ratios of 1.3 : 1 were obtained. As the activity of PGE_2 as a renal vasoconstrictor agent is considerably greater than that of $PGF_{2\alpha}$ (ref. 3), changes in patterns of synthesis of PGs without changes in their rate could influence vascular reactivity.

In contrast to the above findings, important differences were found between the kidneys of GH and normotensive rats in their abilities to degrade PGs to their 15-keto metabolites, the first and most important step in the metabolism of renal PGs[21]. This assay depends on the oxidation of ^3H-PGE_2 by an enzyme, PG 15-hydroxydehydrogenase (PGDH), the activity of which is associated with a high speed supernatant fraction obtained from renal homogenates. Blackwell, Flower and Vane[18] reported that the oxidation of ^3H-PGE_2 by the enzyme obtained from this fraction was linear for the first 5 min during which time approximately one-half of the labelled substrate was metabolised. We have confirmed these findings. A marked reduction of PGDH activity in the kidneys of GH rats was demonstrated when compared to those of normotensive rats (Fig. 4) These differences in PGDH activity were evident when kidneys of normotensive rats of either similar weight or age to those of GH rats were used. Since an age-dependent difference in PGDH activity has been described

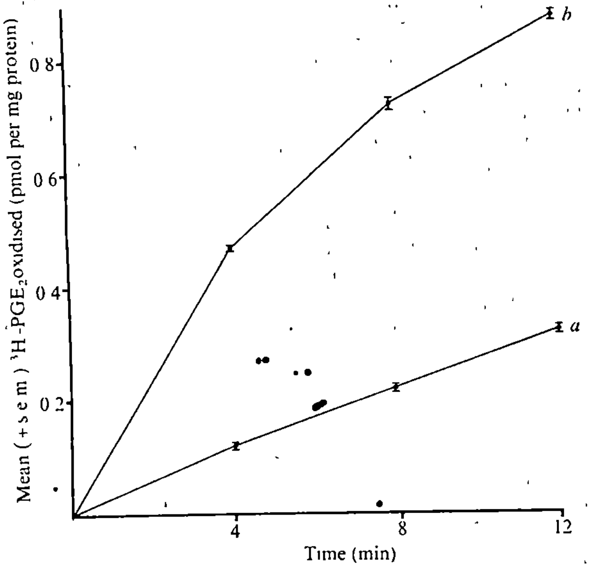


Fig. 4 Comparison of the activity of PGDH from the kidneys of GH (*a*) and normotensive (*b*) rats of similar weight ($n = 3$ for each point). Kidneys of male rats were homogenised in ice-cold 100 mM Tris buffer, *p*H 7.5. After centrifugation at 1,000*g* to remove cellular debris, the supernatant was recentrifuged at 100,000*g* for 1 h in a Beckman Spinco ultracentrifuge. Of the resultant supernatant, 1 ml containing the PGDH was added to a reaction mixture containing 280 pmol NAD^+, 0.1 µCi ^{3}H-PGE_2 and 28 pmol unlabelled PGE_2. After incubation at 37 °C for 4, 8 and 12 min the reaction was stopped by acidification to *p*H 3 with 1 N HCl. A control mixture containing boiled supernatant was similarly treated. Unreacted PGE_2 and its metabolites were extracted by mixing with 1.5 ml ethyl acetate. The phases were separated by centrifugation and 1 ml of the organic layer was removed and evaporated in a vacuum desiccator. The dried residue was then reconstituted in 30 µl of chloroform; methanol (2:1) with unlabelled PGE_2 and 15-keto PGE_2 as markers. This was then spotted quantitatively on plastic-backed silica–gel plates and developed in an ethyl acetate–water–iso-octane–acetic acid (11:10:5:2) system. The zones corresponding to the authentic prostaglandin markers were indicated by brief exposure to iodine. These zones were then cut out and placed in a scintillation vial containing 10 ml Diatol. The radioactivity in each zone was estimated by liquid scintillation counting on a Beckman LS-150 scintillation counter. c.p.m. were converted to d.p.m. using a quench correction curve and external standards. Results are expressed as the mean oxidation of PGE_2 to 15-keto PGE_2 per mg protein (± s.e.m.) with time. Protein content of the supernatant fraction was determined by the Biuret method.

by Pace-Asciak[22] it was important to use as controls age-matched kidneys to characterise changes in renal vascular reactivity. A decreased catabolism of PGs could lead to increased levels within the kidneys of GH rats, and thereby partially explain the enhanced renal vascular responsiveness to noradrenaline and the greater lowering of renal vascular sensitivity to noradrenaline produced by inhibition of PG synthesis in this species (Fig. 3*b*, *c*) PGE_2 released by noradrenaline potentiates the vascular action of the amine as well as having an additive vasoconstrictor action of its own. The latter is shown when large amounts of PGE_2 are infused in the kidney[3]. We obtained evidence supporting the uniqueness of the deficiency of PG metabolism in the GH rat by showing that another form of hereditary hypertension in the rat[23], the Japanese spontaneously hypertensive (SH) rat, was not associated with decreased renal PGDH activity (Table 1). Further, hypertension produced by constriction of one renal artery and of comparable severity to that in the GH rat, did not result in a similar derangement of PG metabolism in either the constricted or the untouched contralateral kidney. In three experiments oxidation of PGE_2 by PGDH was measured in kidney homogenates of renal hypertensive and normotensive rats. Hypertension (approximately 170 mmHg), was produced 5–7 weeks after constriction of the right renal artery using a silver clip (diameter 0.23 mm) leaving the contralateral kidney untouched. The two kidneys were assayed for PGDH activity separately. Rats, similarly treated, which had not developed hypertension were used as controls. Differences in enzyme activity observed between the two types of kidney were small relative to those of GH and normotensives. The finding that normal renal PGDH activity occurs in rats with other types of hypertension suggests that the fault occurring in GH kidneys is not secondary to the high blood pressure.

If lower than normal degradation of PGs contributes to enhanced renal vascular reactivity in the GH rat, then

Table 1 Prostaglandin metabolism in normotensive and hypertensive rats

	Catabolism			Synthesis pmol PG per min per g kidney
	fmol PGE_2 per min per mg protein			
	GH	GH	SH	GH
Hypertensive	76.2±2.9	46.0±6.0	397.3±5.7	17.29±1.26
Normotensive	*201.2±3.3	*222.3±7.1	‡378.6±1.6	17.20±1.36
		†118.3±7.1	305.3±17.9	

Kidneys of male rats were homogenised in ice-cold 100 mM Tris buffer, *p*H 7.5. To the homogenates were added 20 µg each of PGE_2 and $PGF_{2\alpha}$ to saturate the metabolising enzymes. Prostaglandin synthesis was determined using a radiochemical assay. 1 ml of the homogenate was added to the reaction mixture containing 0.1 µCi ^{14}C-arachidonic acid 33 pmol unlabelled substrate, 10 µl reduced-glutathione, 5 mM, and 10 µl (−)-adrenaline, 5 mM. The reaction mixture was then incubated at 37 °C for varying times between 2 and 10 min, and the reaction was stopped by boiling for 2 min. A sample with a boiled enzyme preparation was used as control. The mixture was then acidified and the unreacted arachidonic acid and its products extracted and chromatographed on a silica gel TLC plate (Uniplate) with unlabelled PGE_2 and $PGF_{2\alpha}$ as markers. The zones corresponding to the prostaglandins were scraped into a scintillation vial and the radioactivity determined (as for Fig. 5). The units of enzyme activity, fmol per min per mg protein or pmol per min per g kidney, were calculated from the difference in d.p.m. of each product relative to that of the control, the specific activity of the ^{14}C-arachidonic acid or the ^{3}H-PGE_2, the percentage extraction of each product, the aliquot ethyl acetate taken, the incubation time and the protein concentration or weight of the kidney.

$n = 3$–5

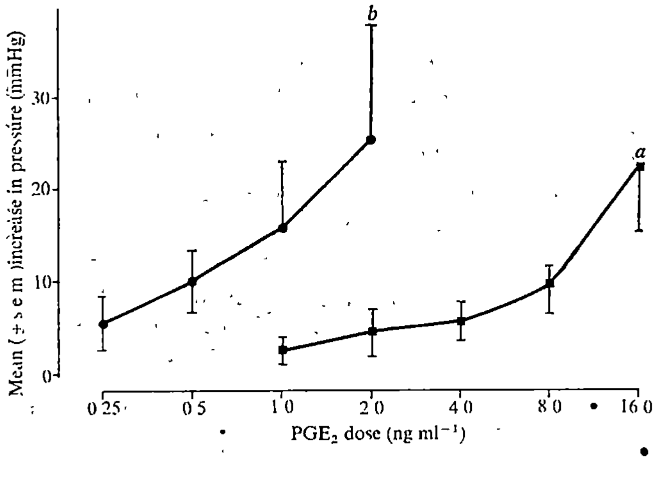
*Weight-matched normotensives.

†Age-matched normotensives.

‡Japanese normotensives.

GH, New Zealand genetic hypertensive; SH, Japanese spontaneous hypertensive.

Fig. 5 Mean changes in perfusion pressure produced by PGE_2 infused for 8 min into kidneys of normotensive (a, $n = 9$) or hypertensive (b, $n = 9$) rats *in vitro*. Curves of hypertensives were found to be to the left of normotensives denoting greater vascular sensitivity to the PG.



it should be possible to show greater than normal vascular reactivity to exogenous PGE_2 in kidneys from these animals. We, therefore, infused PGE_2 in different concentrations and showed increased vascular sensitivity to PGE_2 in the kidneys of GH rats, as indicated by a lowering of the dose required to produce a threshold response (Fig. 5). There was also an approximately tenfold increase in responsiveness to its vasoconstrictor action when compared with its effects on the renal vascular bed of normotensive rats.

Prohypertensive activity

Rats with hereditary forms of hypertension have so far been considered as good animal paradigms of human essential hypertension[19]. Abnormalities of a system involved in blood pressure regulation have long been sought in genetically hypertensive rats[2]. The vasoconstrictor effects of endogenously released PGs coupled with a relative lack of the inactivating enzyme, offer a biochemical basis for hypertension in the GH rat. At the same time, the fact that PGE_2 is vasodilator in the kidneys of most other species must call into question the use of hypertensive rats as a model of human disease.

Prostaglandins as modulators

As modulators, prostaglandins may amplify or attenuate the vascular actions of other hormones or neurotransmitters in concentrations which do not affect the vasculature directly. Thus, prostaglandins have been shown to amplify the renal vascular action of kinins[24] and attenuate those of angiotensin II and noradrenaline[7,13,15]. There is an additional and less well known corollary of possible interactions between modulators and hormones, and one which has bearing on the concept that hypertension may develop in the face of normal activity of either the adrenergic nervous system or the renin–angiotensin system[25]. That is, that an abnormality of the modulator, by itself, could result in elevated blood pressure and we suggest this is the case in the GH rat. Such an explanation accounts for the demonstration that, although the adrenergic nervous system is essential for maintaining elevated blood pressure in GH rats, there is no direct evidence for its activity being increased in this species. Thus, the primary or initiating event would be increased levels of PGE_2, or a similar product of PG synthetase, which results from a deficiency of the major catabolising enzyme in the face of normal rates of prostaglandin synthesis. As emphasised above, it is likely that this disturbance need only occur in the kidney for the development of hypertension. Recent studies on other visceral vascular beds of the rat, demonstrate, however, that one or more products of prostaglandin synthesis[26,27] contributes to the vasoconstrictor actions of adrenergic stimuli The present study is the second which indicates a genetically determined defect in prostaglandin metabolism, the first being a deficiency in synthesis demonstrated in the platelets of a patient with an haemostatic defect[28].

Received November 27, 1975; accepted February 26, 1976

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Early Mesozoic rifting and fragmentation of the Cordilleran orogen in the western USA

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New data make possible the precise definition of the truncated edge of the southern part of the Cordilleran orogen in California, and lead to the identification of possible 'missing pieces' removed during the early Triassic. A probable reconstruction of the Palaeozoic Cordilleran orogen involving large scale lateral transport of major tectonic units is suggested.

MANY lines of evidence indicate that only part of the Palaeozoic Cordilleran orogen is preserved intact in the western USA. An inferred south-western segment of unknown dimensions that presumably extended south-west of California has been lost. North-west-trending Mesozoic arc-trench complexes developed adjacent to the raw edge of a truncated Palaeozoic orogen[1–5]. Few people have attempted to reconstruct the ancient orogen or to identify its dispersed fragments, although studies in the Canadian and Alaskan Cordillera have begun to suggest that certain Palaeozoic terrains may have originated far to the south[6,7], perhaps even adjacent to the truncated margin of the US Cordillera[6].

Before missing parts of the Palaeozoic orogen can be identified confidently and restored to their original positions, it is necessary to bring into closer focus the truncated margin itself. Where is the edge, and what is the nature of the truncated terrains? If these questions can be resolved, it should be possible to specify the nature of the missing parts and, if they can be identified elsewhere, to outline a broad kinematic history of breakup of the orogen.

This article is composed of three parts. In the first part, I discuss current views of the relations of Palaeozoic terrains of northern California and Oregon to their counterparts in Nevada. New data from the Sierra Nevada region of California and their bearing on the location of the truncated edge of the orogen are then presented. In the second part, I present arguments concerning the identity of displaced terrains. I conclude, in the third part, with a possible tectonic reconstruction and model for the truncation and fragmentation of the orogen during early Mesozoic time.

Accepted model of late Palaeozoic orogen

Several reviews envision island arc complexes in the northern Sierra Nevada and eastern Klamath Mountains of California, together with presumably equivalent units in eastern Oregon, as having fringed the Palaeozoic North American continent much as Cainozoic island arcs in the western Pacific festoon the south-eastern margin of Asia. The volcanic archipelago was flanked to the south-east by contemporaneous deep-water chert–argillite facies with minor limestone and mafic volcanic rocks that apparently accumulated in a marginal basin which separated the arc complex from the Cordilleran miogeocline. These marginal basin rocks were thrust south-easterly over shallow-water shelf facies (Fig. 1) during periods of contraction of the marginal basin[4,5,8–10].

This model is evidently appropriate for two distinct parts of the Palaeozoic. (1) Beginning in the Devonian, an arc developed on ultramafic basement in the eastern Klamath Mountains[4,5,10] (1, Fig. 1) and on metasedimentary basement in the northern Sierra Nevada (2, Fig. 1), adjacent to what had formerly been a stable continental margin[10]. The presence of bluechist in the heart of the structurally complex north-westernmost part of the eastern Klamath Mountains[11] (3, Fig. 1), where rocks of Ordovician, Silurian and Devonian ages occur, suggests that the north-east-trending Palaeozoic arc complex (1, Fig. 1) was paralleled by a north-east-trending subduction complex, and therefore faced north-west.

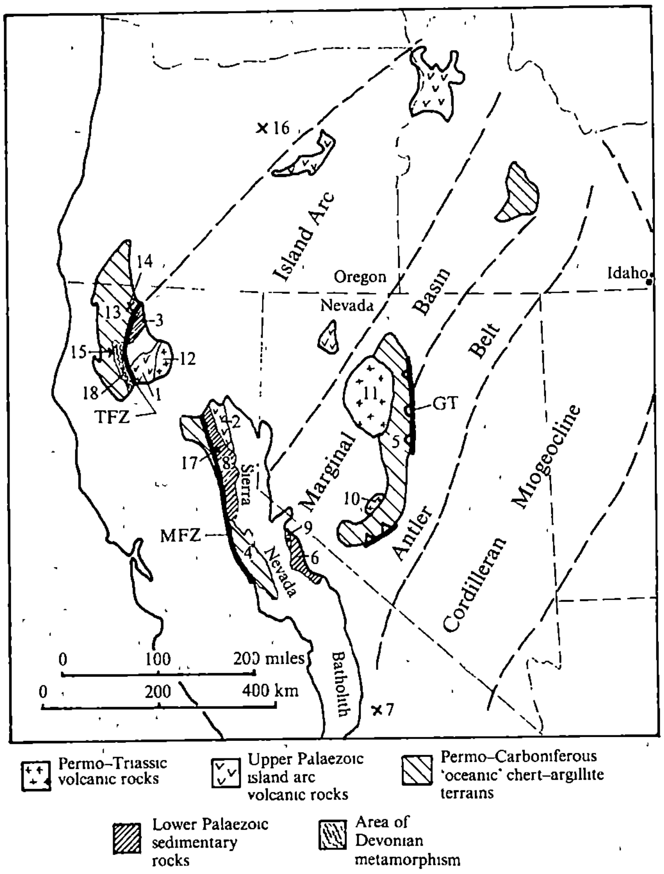
Most workers have concluded that the arc terrain was flanked by a marginal basin to the south-east, but it is important to note that the marginal basin did not originate by back-arc spreading[5,8,9]. During the late Permian–early related arc magmatism, well removed from the continental edge. The sedimentary fill of this trapped marginal basin was uplifted and thrust south-eastward on to the miogeoclinal prism as the marginal basin was closed during the late Devonian–early Mississippian Antler orogeny[4,5].

(2) During Permo–Carboniferous time, an analogous arc-marginal basin system developed in nearly the same position as the earlier couplet, but this time the marginal basin grew by back-arc spreading[5,8,9]. During the late Permian–early Triassic Sonoma orogeny the sedimentary contents of the marginal basin were again uplifted and thrust on to the Antler orogenic belt[4,5,8,9].

Effects of the Antler orogeny have been obscured in places by the younger event, and evidence of a Devonian arc complex apparently exists only in northern California. The Permo–Carboniferous record is more complete, making possible more confident recognition of the various tectonic elements in Nevada, California and Oregon. In neither postulated phase of closure of the marginal basin is the fate of the presumed underlying oceanic crust known[4,5,8,9], but one hypothesis suggests that closure was accomplished by contemporaneous subduction of oceanic crust and obduction of sedimentary fill[5,12].

Sierran Palaeozoic rocks and their relation to the orogen

The foregoing model of arc-marginal basin tectonics is based almost entirely on data from north-central Nevada and northern California. A large terrain of Palaeozoic metasedimentary rocks occurs in part between these better known areas, in the western Sierra Nevada (Fig. 1), and is separated from pre-Mesozoic rocks of Nevada by the Mesozoic Sierra Nevada batholith. Although little is known of specific stratigraphic and structural relationships between Palaeozoic rocks on opposite sides of the Mesozoic batholith, there is no evidence that the batholith occupies a major tectonic boundary.



x 16
Island Arc
Oregon
Nevada
Idaho
Basin
Belt
Marginal
Antler
Cordilleran
Miogeocline
Sierra
Nevada
Batholith
14
13
3
15
12
18
1
TFZ
11
GT
5
17
2
8
10
MFZ
9
4
6
x 7
0 100 200 miles
0 200 400 km
Permo–Triassic volcanic rocks
Upper Palaeozoic island arc volcanic rocks
Permo–Carboniferous 'oceanic' chert–argillite terrains
Lower Palaeozoic sedimentary rocks
Area of Devonian metamorphism

Fig. 1 Sketch map showing inferred tectonic elements of the Cordilleran orogen and specific localities, 1–18, discussed in the text; relations in eastern Oregon are uncertain in detail and highly schematic as shown. The map is modified, in part, from Fig. 1 of ref. 8 and Fig. 4 of ref. 4. GT, Golconda thrust; MFZ, Melones fault zone; TFZ, Trinity fault zone.

The Sierran Palaeozoic terrain lies at a high angle to the north-eastern tectonic trends of the Palaeozoic Cordillera, and its southern half (4, Fig. 1) occupies a "back-arc" position analogous to that of probable marginal basin rocks of north-central Nevada (5, Fig. 1; discussed below). The part of the Sierran terrain north of about latitude 39° is here considered older sedimentary basement of the above-mentioned island arc complex. Recent field studies[13–15], discussed below, have revealed major lithological and structural contrasts between the southern part of the Sierran belt (known as the Calaveras Formation) and the northern part of the belt (to which the name Shoo Fly Formation is applied).

The Calaveras Formation (4, Fig. 1) is a structurally complex assemblage of argillite, chert, subordinate quartzite and greenstone, with several large, lenticular masses of strongly recrystallised limestone. Recent work (refs 13, 14 and my unpublished data) indicates that the Calaveras Formation consists of two major units. The lower unit is a very thick, extensive mass of chaotic submarine slide deposits consisting primarily of fragments and blocks of chert in an argillaceous matrix. Most exposures can be termed diamictite, and bedding is only rarely preserved. The chaotic rocks were deposited on a structurally coherent sequence of mafic pillow lava, pyroclastic rocks, and interbedded shale. These rocks are overlain on the east by an upper sequence of quartzites (ref. 16 and my unpublished data) in which graded beds indicate tops to the east, and interbedded olistostromes. All rocks of the Calaveras Formation have been penetratively deformed, producing an east- to north-east-plunging extension lineation and a regional schistosity. The Calaveras Formation may be Permo–Carboniferous, but faunal evidence is meagre and does not preclude the existence of rocks of other ages. The penetrative deformation predates the development of Jurassic structures and the intrusion of Jurassic plutons; and may be late Palaeozoic or early Mesozoic[13,14].

As Speed suggested[17], the Calaveras Formation is probably correlative with the Havallah sequence in north-central Nevada (5, Fig. 1), which has been interpreted by other workers to have accumulated in the late Palaeozoic marginal basin[4,5,8,9]. My reconnaissance of parts of the Havallah sequence has revealed the existence of widespread submarine slide deposits consisting primarily of chert–argillite diamictite with assorted blocks of chert, sandstone, limestone and greenstone. In addition, evidence of penetrative deformation is present. The lower chert–argillite–greenstone units of the Havallah sequence are overlain by an upper quartzitic sequence[18,19]. These highly distinctive lithological and structural characteristics are identical to those of rocks of the Calaveras Formation. The Havallah sequence contains fossils of middle Pennsylvanian to early Permian age[8]. During the Sonoma orogeny, these marginal basin sedimentary and volcanic rocks were thrust south-eastward along the Golconda thrust on to rocks of the Antler orogenic belt[4,5,8,9,17].

Owing to their analogous palaeogeographic setting, remarkably similar lithology and structure, and probably similar age[17], I suggest that the chaotic chert–argillite facies and quartzites of the Calaveras formation are part of the marginal basin assemblage represented by the Havallah sequence. The chaotic nature of parts of both sequences probably resulted from repeated slumping in this tectonically unstable environment; shallow-water limestones in both areas were introduced into deep-water environments by large scale slumping. The penetrative deformation of both terrains probably represents shortening during the Sonoma orogeny when the marginal basin was partially closed. Rocks representative of the late Palaeozoic marginal basin can thus be considered to extend as far west as the Melones fault zone in the western Sierra Nevada (Fig. 1).

Like the Havallah sequence, rocks of the Calaveras Formation may be largely allochthonous; and if so they are separated from miogeoclinal Palaeozoic rocks of the eastern and southern Sierra Nevada (6, Fig. 1) by the projected Golconda thrust, whose actual trace has been obliterated by the Sierra Nevada batholith. The Golconda allochthon may in addition include part of the Garlock Formation (7, Fig. 1) as suggested by Burchfiel and Davis[4,5], requiring projection of the thrust 300 km south of its known extent in north-central Nevada.

Metasedimentary rocks in the northern half of the Sierran belt (8, Fig. 1) are known as the Shoo Fly Formation[20,21]. These rocks are considerably older than the Calaveras Formation and may be Silurian or older[15,16,21]. They are unconformably overlain by Upper Devonian volcanic rocks[22,23] (2, Fig. 1) which form the basal part of the volcanic arc discussed above[2,4,5,8–10]. The Shoo Fly is made up of slate, phyllite, graywacke and distinctive quartzose sandstone, bedded chert and rare thin limestone beds[15,21,23]. Its thickness is unknown as it is tightly folded and penetratively cleaved.

The Shoo Fly comprises a thick, deformed metasedimentary pile on which the Devonian–Permian arc complex developed[2,4,5,8–10], and thus contrasts with the ultramafic basement of the arc in the Klamath Mountains. It resembles lithologically the siliceous facies[10] of the early Palaeozoic eugeocline to the south-east. The entire northern Sierra Nevada terrain and that of the eastern Klamath Mountains, including both younger arc rocks and older metasedimentary and ultramafic basement, may have been rifted north-westerly away from the eugeocline during growth of the late Palaeozoic marginal basin to the south-east.

Widespread Permo–Triassic volcanism

Thick sequences of silicic volcanic rocks or probable Permo–Triassic age occur in many parts of the Cordilleran orogen, within both the former marginal basin and the arc complex proper.

In the eastern Sierra Nevada, the late Palaeozoic Koip sequence[24–26] (9, Fig. 1) consists of andesitic and rhyolitic lava and pyroclastic rocks[26]. Age assignment is based on two Rb–Sr whole-rock isochron ages: 240 Myr (ref. 24) and 250 Myr (ref. 25). These rocks, together with similar rocks of the Permo–Triassic(?) Excelsior Formation[26] within the Sonoma orogenic belt[19,27] (10, Fig. 1), may occupy a structural position analogous to that of the Permo–Triassic Koipato sequence of north-central Nevada (11, Fig. 1), which consists of more than 3 km of rhyolitic and andesitic lava and pyroclastic rocks[8,19]. The Koipato has yielded fission-track and lead-alpha ages that average about 250 ± 40 Myr (ref. 28). Koipato rocks were erupted and deposited on the Havallah sequence, evidently after thrusting[8,17,19]. Although precise age equivalence cannot be demonstrated, the Koip, Excelsior and Koipato collectively

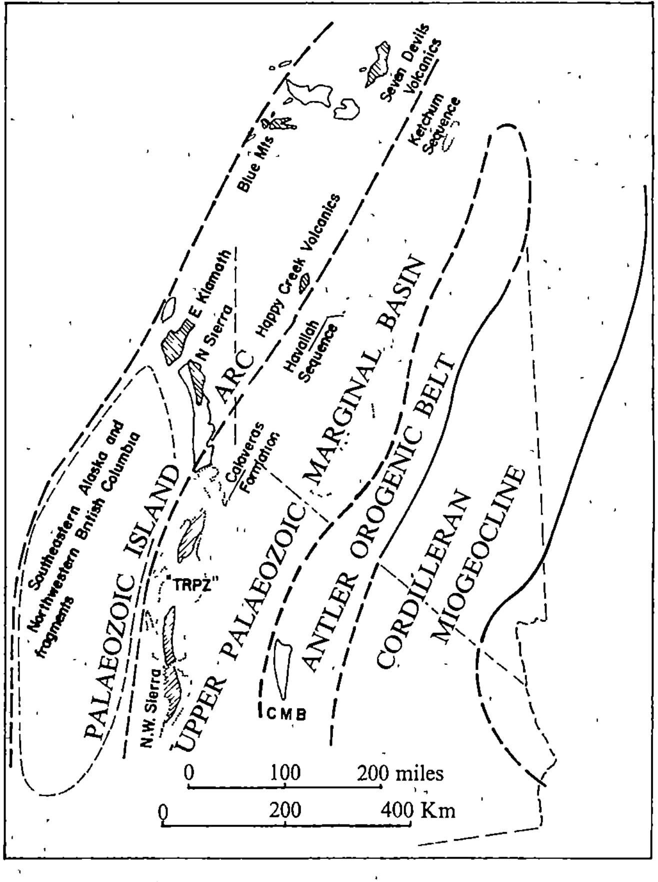


Fig. 2 Proposed tectonic reassembly of southern part of Cordilleran orogen for Permian time, before the Sonoma orogeny, which involved shortening and thrusting of marginal basin fill on to the Antler orogenic belt. Terrains north-east of the line of truncation have been shifted 50 km north-west of their present locations to eliminate effects of the Sonoma orogeny; Mesozoic and Cainozoic displacements have not been treated. Arc rocks of the eastern Klamath Mountains have been shifted 60 km closer to those of the northern Sierra Nevada[53]. The dot pattern outlines chert–argillite terrains which were once part of the marginal basin. North-east ruling marks probable remnant arcs within the marginal basin; north-west ruling marks arc volcanic sequences in the autochthonous part of the Palaeozoic island arc. TRPZ, western Palaeozoic and Triassic belt; CMB, central metamorphic belt. See text for discussion.

indicate extensive Permo–Triassic silicic volcanism within the Sonoma orogenic belt.

Notable Permo–Triassic volcanic rocks much like the above units are found, in addition, in the eastern Klamath Mountains, overlying Devonian to Permian arclike volcanic rocks discussed earlier (12, Fig. 1). These units, the Dekkas Andesite and Bully Hill Rhyolite, constitute up to 2 km of lava and pyroclastic rocks[29], and perhaps should be regarded simply as continued arc volcanism. Nevertheless, the widespread Permo-Triassic andesitic and rhyolitic volcanism within both former arc and marginal basin settings may be indicative of a transition to extensional tectonics following the Sonoma orogeny[17], preliminary to rifting within the orogen. Burchfiel and Davis[5,12], however, consider such volcanism to be arc volcanism which resulted from subduction of the oceanic crust on which the Havallah sequence was presumably deposited[8].

Truncated edge in central California

As shown here, recognisable elements of the late Palaeozoic Cordilleran marginal basin continue south-westerly across the Sierra Nevada batholith into the western Sierra Nevada. Such rocks do not extend beyond the Melones fault zone, the major tectonic boundary of the region, and which apparently represents a collision boundary between the rifted Palaeozoic margin and a Jurassic island arc[15]. The Melones fault zone has been interpreted as a high-angle reverse fault[30], strike-slip fault[31], and as a thrust fault[32]. Limited exposures of melanges with Permian limestones occur west of the Melones fault zone[33], but their Tethyan faunas[34], and tectonic setting within a Jurassic island arc[15], suggest that these rocks did not originate within the Cordilleran orogen. The Melones fault zone, whatever its detailed movement history may be, appears to mark the modified truncated edge of the Palaeozoic orogen in central California. On tectonic grounds, its probable northward extension is viewed as the Trinity fault zone[32] (Fig. 1), which, as shown here, truncates the north-east-trending arc and subduction terrains of the eastern Klamath Mountains and abruptly juxtaposes them against arcuate, northerly trending terrains to the west.

The missing pieces

Tectonic elements presumably missing from the Palaeozoic orogen are the south-western continuation of the marginal basin and the remainder of the Devonian–Permian arc complex. I assume that these missing pieces had some finite areal extent and might be recognised in some other geological setting.

Is the western Klamath terrain a marginal basin fragment? The western Palaeozoic and Triassic belt of the Klamath Mountains[35] (13, Fig. 1), like the Calaveras and Havallah terrains, is underlain largely by deformed chert–argillite, mafic volcanic rocks and lenses of limestone[35–37]. Large parts of the belt are chaotic[38,39] and closely resemble diamictite of the Calaveras Formation and Havallah sequence (my observations made in 1972–75). Most fossils from limestone lenses within the southern part of the terrain indicate Pennsylvanian to Permian ages[36], although rare Devonian and Triassic fossils have been reported[36,40]. A probable southern continuation of the Klamath terrain occurs, in addition, west of the Melones fault zone in the north-western Sierra Nevada[15,32].

Rocks of the western Palaeozoic and Triassic belt have been interpreted as part of a late Palaeozoic subduction complex related to the late Palaeozoic arc of the eastern Klamath Mountains and northern Sierra Nevada[2,4,5], invoking blueschists in two areas near the eastern edge of the belt[4,5,11,37] (14, 15, Fig. 1). For the following reasons this interpretation is considered untenable.

(1) As argued earlier, the probable north-east-trending subduction complex related to the late Palaeozoic arc (1, Fig. 1) lies in the north-western part of the eastern Klamath Mountains. (2) The structural trends of the western Palaeozoic and Triassic belt and its southern continuation parallel the Trinity and Melones fault zones which mark the truncated edge of the orogen and are highly discordant with north-east-trending arc terrains. (3) Available evidence indicates that deformation and metamorphism of the sedimentary rocks was post-Permian and pre-late Jurassic[39,41]. (4) Blueschists in the eastern part of the western palaeozoic and Triassic belt have yielded a 215-Myr K–Ar date[5], which allowing for uncertainties in the Triassic part of the time scale[42], could be as young as late Triassic. These blueschists are clearly part of an inner, early Mesozoic blueschist belt in the Cordillera, including localities in central British Columbia[43], the Northern Cascades[44], east-central Oregon[45] (16, Fig. 1), and undated lawsonite blueschist along the Melones fault zone in the northern Sierra Nevada (ref. 54) (17, Fig. 1). Published K–Ar ages on blueschists in this belt all cluster around 210–220 Myr[5,43,44]. This and the geological setting of these blueschists strongly indicates that they formed in subduction zones related to Upper Triassic and Lower Jurassic arc rocks in eastern California, western Nevada, Idaho and British Columbia[42,47–49].

I believe that the rocks of the western Palaeozoic and Triassic belt are best interpreted as part of an already chaotic terrain that originated elsewhere and may have been subducted during the early Mesozoic with the local production of blueschist. I suggest (Fig. 2) that the western Palaeozoic and Triassic belt represents part of the missing marginal basin sequence and has been shifted some 500–600 km north-west from its original position opposite the Calaveras Formation after the truncation event. This interpretation accounts for the fact that the apparently correlative rocks of the Calaveras Formation and western Palaeozoic and Triassic belt lie on opposite sides of the Trinity–Melones fault zones (Fig. 1), and also allows for the existence of blueschist in the Klamath terrain and its southern continuation and its apparent absence in the Calaveras Formation[13,14,54] (also my unpublished data).

Andesitic rocks flanked on both sides by ophiolitic rocks, recently noted in the western Palaeozoic and Triassic belt[36], may represent part of a remnant arc which became isolated by continued spreading within the marginal basin Volcanic rocks within parts of the terrain in Oregon and the north-western Sierra Nevada[50] may also represent parts of remnant arcs, and shreds of ophiolites are probably represented in these areas as well.

The north-north-west-trending central metamorphic belt (18, Fig. 1), a tectonic sliver of hornblende schist and overlying mica schist[37,51] between the western Palaeozoic and Triassic belt and the Trinity fault zone, has late Devonian to Mississippian K–Ar and Devonian Rb–Sr ages[41]. Burchfiel and Davis[4,5] regard this terrain as the subduction complex related to Devonian arc activity to the east although blueschists are lacking, and the Trinity fault zone juxtaposes the central metamorphic belt directly against the south-western part of the north-east-trending arc complex (Fig. 1). It could alternatively be an exotic fragment[2], but as Burchfiel and Davis[4,5] point out, its metamorphic ages suggest some relation to the Antler orogeny A highly speculative possibility, adopted in Fig. 3, is that rocks of the central metamorphic belt represent a deep-seated southerly portion of the Antler orogenic belt rifted away from its original position together with rocks of the western Palaeozoic and Triassic belt. The westward vergence of synmetamorphic overturned folds within the terrain[4,5,51], however, is difficult to explain unless it is attributed to eastward subduction beneath the Antler orogenic belt[5,12]. Unfortunately, blueschists are lacking. Whatever its origin, the central metamorphic belt now forms a thrust plate that has overridden the western Palaeo-

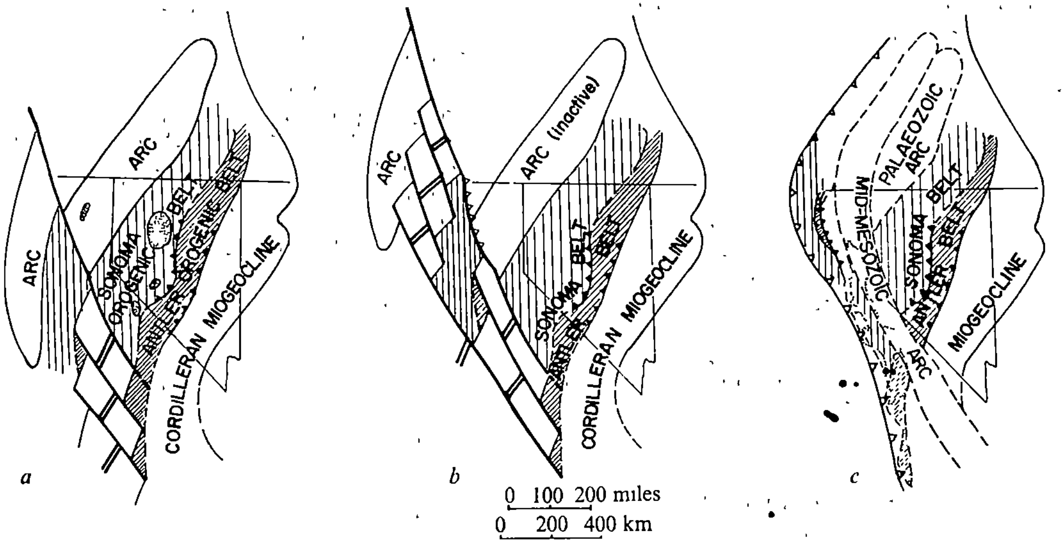


Fig. 3 Tectonic diagrams showing proposed sequence of events leading to present distribution of Palaeozoic terrains in the Cordilleran orogen. *a*, Stage of fragmentation during early Triassic. Stipple pattern shows areas of Permo–Triassic silicic volcanism. *b*, Hypothetical stage of fragmentation during middle Triassic. Arc and marginal basin fragment are separated by jump in plate boundary; marginal basin fragment begins to underthrust truncated edge of arc. *c*, Initiation of subduction along truncated margin during the late Triassic. Partial subduction of marginal basin fragment produces local blueschist metamorphism in Klamath Mountains and Northern Sierra, Nevada. Late Triassic to mid-Jurassic arc is superimposed on older tectonic elements. The dot pattern shows future site of Upper Jurassic–Upper Cretaceous Sierra Nevada batholith.

zoic and Triassic belt for 20–30 km, and has itself been overridden by rocks of the eastern Klamath belt for distances of 30 km or more[51].

I next consider a possible arc fragment in the Pacific North-West. Figure 2 depicts the original south-western extent of the late Palaeozoic volcanic arc and marginal basin which fringed North America before the truncation event. The western Palaeozoic and Triassic belt and its Sierran continuation are shown as the south-westerly part of the marginal basin. Local remnant arcs are also shown.

The remaining part of the orogen to be identified is the corresponding arc segment which must originally have lain along the north-western flank of the marginal basin. Monger and Ross[7] have suggested that Upper Palaeozoic rocks of the Coast Mountains of British Columbia and south-eastern Alaska may be far-travelled from their original sites of accumulation. Jones and others[6] argued that the Lower Palaeozoic rocks of south-eastern Alaska, at least, were derived from the truncated margin of the Cordilleran orogen, probably in northern California. Palaeozoic sequences containing volcanic rocks of basaltic to rhyolitic composition occur widely within this 1,500-km-long region and rest on a basement of metavolcanic and ultramafic rocks[6,9].

It seems likely that the Palaeozoic rocks in this region in part represent a volcanic arc complex that developed on lower Palaeozoic basement in late Palaeozoic time[7]. Much of the terrain, especially its northern part, can be viewed as the missing arc segment required by the arguments presented here, in turn satisfying the palaeontological contraints invoked by Monger and Ross[7]. If so, the puzzle is relatively complete (Fig. 2).

Possible sequence of fragmentation

Figure 3 shows hypothetical stages of fragmentation of the orogen, resulting in dispersal of its component parts Figure 3*a* shows the initiation of separation, which perhaps began in the early Triassic, after the Sonoma orogeny[4,5], when silicic volcanism was widespread within the orogen. Figure 3*a* shows a rhombochasm, like the Gulf of California, forming behind the arc and marginal basin fragment.

Figure 3*b*, approximately middle Triassic, shows two significant changes: a second rhombochasm begins to separate the arc segment from the Palaeozoic marginal basin fragment. There is no direct evidence of timing, but some such mechanism is required for the separation of the arc and marginal basin fragments. The marginal basin fragment perhaps impinged obliquely against the blunt end of the arc thus retarding its motion, and producing thrusting along its boundary.

Figure 3*c*, drawn for late Triassic to early Jurassic time, shows that the marginal basin fragment has underthrust the truncated end of the arc with the initiation of north-eastward subduction of lithosphere beneath the newly reshaped continental margin. This subduction resulted in the production of blueschists and the generation of a new magmatic arc at a high angle to the Palaeozoic trends[4,5,15,46]. The arc fragment has by this time progressed on its way to south-eastern Alaska.

Implications and conclusions

The model described here has several attractive features. It provides a plausible context for assembly of the various Palaeozoic terrains of the Klamath Mountains, one that seems more satisfactory than repeated collisions of exotic plates[2]; it is consistent with known ages of deformation and metamorphism, and it provides some insights into the basic similarities and differences between Palaeozoic terrains in the Klamath Mountains and the western Sierra Nevada of California In addition, it is consistent with tectonic inferences drawn from studies of the Canadian and Alaskan Cordillera[6,7].

If this model has any validity, it seems to imply that western North America was repeatedly subjected to major north-westerly transcurrent movements, beginning about 850 Myr ago, with north-west–south-east rifting which initiated the Cordilleran geosyncline[52], continuing with the early Mesozoic movements suggested here, and again in the Cainozoic, with slicing and north-west tectonic transport related to the San Andreas system.

Because chaotic sequences like the Calaveras, the western Palaeozoic and Triassic belt, and the Havallah sequence may have acquired their chaotic character behind island arcs, with or without subduction, it seems that the chaotic character of assemblages alone may not be sufficient for them to be labelled subduction complexes; other lines of evidence are needed.

The proposed scheme of reassembly and fragmentation is consistent with many known aspects of Cordilleran geology, but is obviously an oversimplification of complex, controversial and as yet poorly known geology. I consider the first part of this article, which locates the truncated edge of the orogen, the most certain. The second and third parts are successively more speculative, and new data will require modification or even rejection of some of the suggestions. I present this analysis as an alternative to the orthodox models of orthogonal subduction and collision tectonics currently in vogue[2,4,5,9]. As noted here, major transcurrent motions along longitudinal faults deserve as much careful consideration as simple subduction in the generation of viable models of the tectonic evolution of Cordilleran mountain belts.

I thank W. Alvarez, D. S. Cowan, I. W. D. Dalziel and W. R. Dickinson for reading the manuscript, and D. S. Cowan and W. R. Dickinson for discussions. This work was partially supported by the Department of Geological Sciences, Columbia University.

Received October 16, 1975; accepted February 26, 1976.

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letters to nature

Observational tests in cosmology

It is often said that the determination of the deceleration term is one of the main aims of observational cosmology. In the following comments this view is questioned, and in a universe of indeterminate density, the third time derivative of the scaling variable is shown to be of greater fundamental importance.

Let $K(=k/R^2)$ be the curvature of a homogeneous and isotropic model of the Universe, where R is the scaling variable, and k is the curvature constant indicating whether the model is closed ($k = 1$) and space is finite, or open ($k = 0, -1$) and space is infinite. According to general relativity, the dynamic equation of a zero-pressure model is

$$K = 4\pi G\rho - H^2(q+1) \qquad (1)$$

where ρ is the average mass density. The Hubble term H and the deceleration term q are

$$H = \dot{R}/R$$

$$q = -\ddot{R}/RH^2$$

and dots denote time derivatives. The cosmological term, which at present cannot be measured independently by any known means, is

$$\Lambda = 4\pi G\rho - 3qH^2 \qquad (2)$$

The verification of the important cosmological equation (1) requires measurements of the quantities K, ρ, H, and q. When, however, (1) is assumed to be valid, the resolution of the issues as to whether the Universe is open or closed, and whether the Λ term is zero or not, requires measurements of ρ, H, and q.

In principle the quantities K, H, and q can be determined, although in practice their precise determination is exceedingly difficult[1]. The determination of the average density ρ is quite another matter—for its exact value cannot be determined, even in principle. The reason for this is obvious. We know approximately the contribution to the average density from luminous galaxies, and can even attempt with tongue in cheek to estimate the contributions from the intergalactic medium and known non-luminous objects such as stellar black holes. But we cannot, of course, include all the unknown contributions (dead galaxies? primordial black holes? . . . ?) that will be discovered in the future.

Cosmological tests of a local nature[2] concerning the sign of K and the value of ρ, based on age determinations, assume first the validity of (1) and second a zero value for the Λ term, and they must therefore be regarded as doubly uncertain.

If in principle the density is indeterminate, then, like the Λ term, it must be removed from (1). In a form analogous to the deceleration term, let

$$Q = \dddot{R}/RH^3$$

be a dimensionless third-order time derivative. The differentiation of equation (1), with ρR^3 kept constant, gives

$$K = H^2(Q-1) \qquad (3)$$

with the auxiliary relationships

$$\Lambda = H^2(Q-2q) \quad (4)$$

$$4\pi G\rho = H^2(Q+q) \quad (5)$$

Thus the removal of ρ also eliminates q from the cosmological equation (3), and the deceleration term is reduced to a subordinate role in equations (4) and (5).

From equation (3) it is seen that verification of general relativity on cosmic scales requires measurements of K, H, and Q, and the Universe is closed (open) when Q is $>$ ($<$) 1. Weinberg[3] has drawn attention to the problem of extracting separately the values of K and q from observational data. Attention is now drawn to an additional problem: until the density is known with greater precision than is possible at present, the value of q is irrelevant for testing the validity of general relativity. Moreover, when general relativity is assumed to be valid on cosmic scales, the traditional observational parameters H and q are inadequate for deciding such questions as: (1) is the Universe open or closed? (2) is the Λ term zero? and (3) is there 'missing mass'? Verification, and the resolution of these issues, depends on the value of Q and therefore $\dot{R}$, and at present, unfortunately, we have no knowledge of this value.

The assumption that the Λ term is zero greatly simplifies the problems of observational cosmology. In this case, $Q = 2q$, and

$$K = H^2(2q-1) \quad (6)$$

and verification requires measurements of K, H, and q, and the Universe is closed (open) when q is $>$ ($<$) 0.5.

Reasons for rejecting or retaining the Λ term are still of a philosophical flavour. A particularly appealing argument proposes that Λ is retained provisionally as a reminder of the possibility of undiscovered cosmic complexity. As an illustration, the Universe consists perhaps mainly of matter and relatively little antimatter[4]. Does this cosmic predilection for matter arise from some quirk in the properties of elementary particles, or does it come in some unknown way from the Λ term and hidden richness in the properties of space-time?

Of the physical systems that range in size from atoms ($\sim 10^{-8}$ cm) to galaxies ($\sim 10^{23}$ cm), electromagnetic interactions dominate on the atomic scale at one extreme, and gravitational interactions dominate on the galactic scale at the other extreme. It is found, on going down five orders of magnitude in size from atoms to elementary particles, that electromagnetic theory is inadequate and is supplemented by weak and strong interactions. Why then, in the absence of observational proof, should we assume that gravitational theory remains unmodified on going up five orders of magnitude in size from galaxies to the Universe? A universe that contains everything is surely as complex as an elementary particle? Considerations of this kind again suggest that the Λ term should be provisionally retained, even though at present we are not aware of its purpose in the cosmic design.

Most observational work in general relativity has in the past been devoted to testing the Einstein equation within stellar-like systems. But we must not forget that confirmation of general relativity in the Solar System and on the scale of stellar systems does not automatically guarantee that the theory is unassailable on the cosmic scale. The Riemannian metric is the most appealing feature of general relativity when applied to cosmology. The identification, however, of the coupled 'matter' tensor solely with energy and momentum has not been submitted to tests on the cosmic scale, and here again is reason for retaining the Λ term until verification is forthcoming. On this subject it is worth recalling Einstein's[5] words: "The right side [the 'matter' tensor] is a formal condensation of all things whose comprehension in the sense of a field theory is still problematic. Not for a moment, of course, did I doubt that this formulation was merely a makeshift in order to give the general principle of relativity a preliminary closed form."

I acknowledge the hospitality of the National Radio Astronomy Observatory operated by Associated Universities Inc., under contract with the National Science Foundation.

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Received February 17; accepted March 3, 1976.

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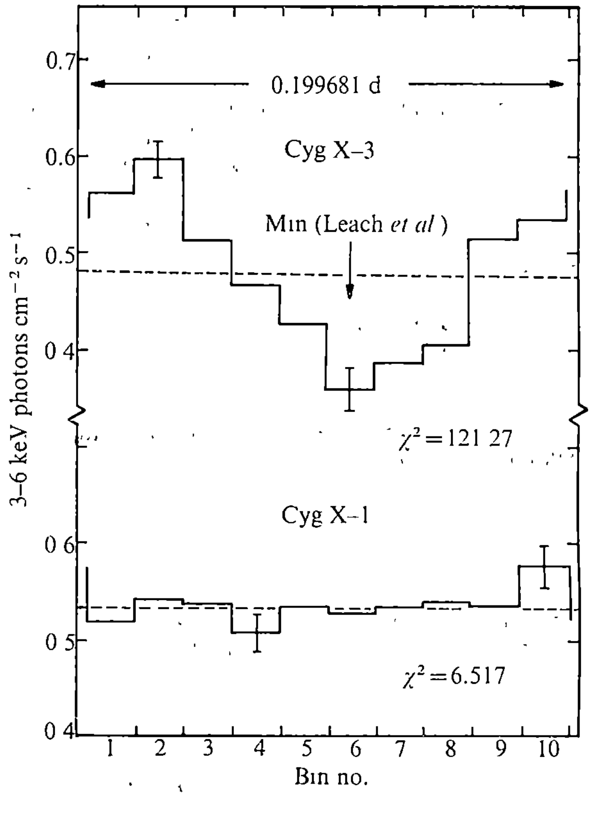
Evidence for a 17-d periodicity from Cyg X-3

Cyg X-3 (3U2030+40) has exhibited phenomena which are observationally unique among identified X-ray sources. The giant radio flare of 1972 is, perhaps, the most spectacular such anomaly, but there are others no less unusual. A wide variation in X-ray spectra has been observed, including the identification of X-ray emission lines at some times, and consistency with a black body at others[1]. The approximately sinusoidal 4.8-h variation[2] is at a period far in excess of any rotation period which has been ascribed to the compact members of other binary sources, and at least four times shorter than any comparable orbital period. Models have been constructed which identify the 4.8-h variation with orbital period[3-5], acknowledging the peculiar geometries which could give rise to a smooth 4.8-h effect in observed X rays. We here present data indicating that a much longer periodicity of ~17 d is also characteristic of Cyg X-3.

The Ariel-V All-Sky Monitor (ASM), from which most of our data are taken, has been described in detail elsewhere[6]. The

Fig. 1 Approximately 100 d of single-orbit Cyg X-3 and Cyg X-1 ASM data obtained between December 1974 and March 1975 folded at the Cyg X-3 period (with indicated phase) of ref 7. The indicated χ^2 values are for 10-bin folds (9 degrees of freedom) against the hypothesis of a constant source intensity.



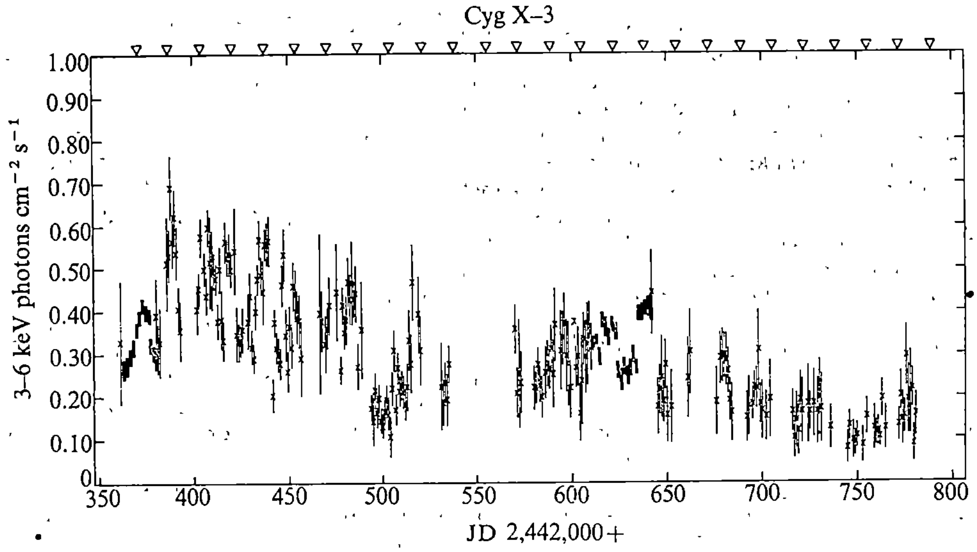


Fig. 2 Continuous record of Cyg X-3. The data points are daily averages of ASM data, with ±1-σ error bars. The solid bars are ±1-σ thick, 1.6-d averages of SSE data. The latter are 2–18 keV measurements which are normalised to the natural ASM ordinate with the response of both instruments to the Crab Nebula. The grid above the data indicates the positions of expected maxima with the best fold values of period (16.75 d) and maximum (JD 2,442,522).

important parameters are an effective pinhole area of 0.6 cm^2 in the energy band 3–6 keV, an average duty cycle for source observation of ~1%, and no temporal resolution finer than 100 min. Typically, ~10 Cyg X-3 counts are accumulated each 100-min orbit in a resolution element of spatial dimensions ~10°×10°, with a background of ~2 counts.

Figure 1 is a useful verification of experiment performance on Cyg X-3. Single-orbit data from ~100 d are plotted |4.8 h| from Cyg X-3 and, as a control, Cyg X-1 (with which it might conceivably be confused with resolution elements centred ~10° apart). Data points are accepted only if they represent an unambiguous determination of source intensity (the possible contribution from other sources is <10%, and the intensity is at least twice the estimated 1-σ error after all corrections have been applied). In folding, the data from each 100-min accumulation are tagged with the orbit midtime. Each bin in Fig. 1 is statistically independent of the others, as an orbit contributes only to the bin that contains its midtime (even though the data are accumulated over the equivalent of 3–4 bins). Both the shape (and phase) are in excellent agreement with the results of ref. 7, indicating that the present measurements are consistent with their period (and error) of 0.1996811±0.0000016 d.

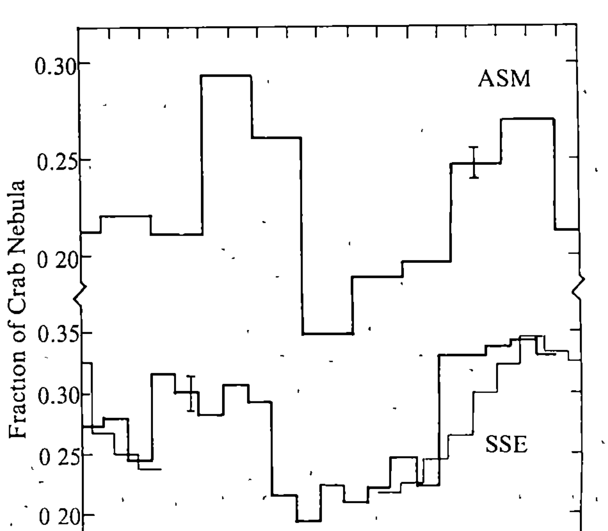
Additionally there is considerable variability in the day-to-day intensity of Cyg X-3, as shown in Fig. 2. This behaviour is in marked contrast to the constant (within the relatively poor statistics) day to day nature of low-state Cyg X-1 data measured simultaneously with the same experiment[8]. There is, however, some indication of regularity in the Cyg X-3 variations on time scales ≳1 week. As the Cyg X-3 data from individual orbits do not always satisfy the 2-σ condition, the ASM data used in Fig. 2 are derived from ~½-d accumulations which are then analysed in exactly the same way as are the individual orbits (they are treated as single long duration orbits). It is not possible unambiguously to compensate completely for the 4.8-h variation, so no attempt has been made to do so. This variation, as well as gaps in the finite data string and an apparently erratic source behaviour, result in many periods in excess of a few days which give relative χ^2 maxima. The effect near 17 d is not only the most significant statistically, but also exhibits a roughly symmetrical χ^2 distribution which has a width commensurate with the length of the data sample. For more than one year's data folded in this way, the best period is 16.75±0.25 d with a phase at maximum of JD 2,442,522±2 (as determined from the folded data, not an individual peak) near the midpoint of the data accumulation interval.

Figure 2 also contains data from the Ariel V Sky Survey Experiment (SSE) against which the 17-d hypothesis may be tested. It is important to note that the latter measurements are obtained in the gaps of the ASM coverage, as the two experiments possess mutually exclusive fields of view. The data are generally consistent with the displayed grid of 16.75 d, but it is clear that the effect is not completely reproducible (as the apparent maxima are not always clearly defined, and are sometimes absent). Nevertheless, the general agreement of the SSE data with the ASM grid is independent evidence for the reality of the candidate period.

Figure 3 contains the same ASM data folded |33.5 d|, or precisely twice the best value of period. The lower trace is the SSE data plotted in phase with the upper trace. Attempts to test the 16.75-d hypothesis with older data in the literature are inconclusive (as these are essentially point measurements), but it is interesting to note that the highest intensity value of Cyg X-3 reported from Uhuru in ref. 7 is in phase with the ASM maxima.

In ref. 1, the authors pointed out that the "high intensity state" of Cyg X-3 is relatively well fitted by a structureless black body, in contrast to the considerably more complex spectra observed in lower intensity states. They further suggest that the total source luminosity is close to the Eddington limit, and approximately constant, regardless of spectral form. This interpretation of the "high intensity state" is, therefore, a manifestation of the relatively better efficiency of contemporary experiments near the black-body peak than at higher energies. We are assuming here that this interpretation is correct, and

Fig. 3 ASM data for >1 yr folded at 33.5 d (upper trace), and SSE data plotted in phase (lower trace). Both are normalised with their respective responses to the Crab Nebula.



that the times of Cyg X-3 maximum correspond to increased electron scattering in the source.

One possible explanation would arise naturally if the 17-d effect was the orbital period of the binary system containing Cyg X-3. In this case, however, the stability of the 4.8-h variation (which would now be interpreted as a slow source rotation) would seem to constrain this hypothesis severely. No apparent 17-d Doppler variation in the 4.8-h modulation is detectable in our data ($v_x \sin i < 300\ \mathrm{km\ s^{-1}}$) and it is difficult to account for the long term stability of this period unless the surface field is much lower than that expected if the observed 4.8-h modulation arises from rotation.

A less drastic suggestion (one which does not alter the identification of 4.8 h with the orbital period) is that the 17-d effect is analogous to the 35-d variation in Her X-1 (see ref. 9). The consistency of both Cyg X-3 and Her X-1 with contact-binary models (in contrast to the supergiant–stellar-wind models reconcilable with the mass source in other identified X-ray binaries) makes this conjecture attractive. It is interesting to note that the interpretation of this effect in terms of free precession[10,11] does not necessarily require a neutron-star source for Cyg X-3 just because the 17-d and 35-d time scales are comparable. A precession period of 17 d is entirely consistent with either a neutron star with rotation period ~1 s, or a white dwarf having a rotation period of the order of minutes. Its interpretation in terms of the precession of the primary member of the binary system (compare ref. 12) is likewise insensitive to the nature of the secondary. While it does not appear that the 17-d effect can unambiguously distinguish between a white dwarf and a neutron star, the inferred high luminosity and relatively hard spectrum out to ~40 keV would seem to favour the latter.

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Satellite observations of planetary waves in the mesosphere

The pressure modulator radiometer (PMR) is one of the instruments carried by the Nimbus 6 observatory satellite launched on June 12, 1975 into a near polar circular orbit at an altitude of ~ 1,000 km. The PMR sounds atmospheric temperature in the upper stratosphere and mesosphere (the 40–95 km height range) by measuring infrared emission from atmospheric carbon dioxide in its 15-μm band[1]. With this instrument measurements of mesospheric temperature with global coverage are being made for the first time. Here we present some of the first results from the PMR, which show, in particular, the propagation of a planetary wave from the troposphere up to the mesopause.

The radiation to be detected is passed through a cell of carbon dioxide which acts as a filter and in which the cell pressure is varied sinusoidally at ~ 15 Hz. The amplitude of the resulting signal selected at this frequency is a measure of the intensity of incident radiation from very narrow spectral intervals near each line centre. This radiation originates in the mesosphere, so the PMR provides a method of measuring the temperature at mesospheric levels. This basic technique[2,3] has been used in the Nimbus 4 and 5 selective chopper radiometers (SCR)[4,5] which measure emission up to ~ 55-km altitude by chopping between different cells of carbon dioxide. By varying the pressure in a single cell, problems of mismatch between cells are avoided and temperature can be sounded to much higher levels.

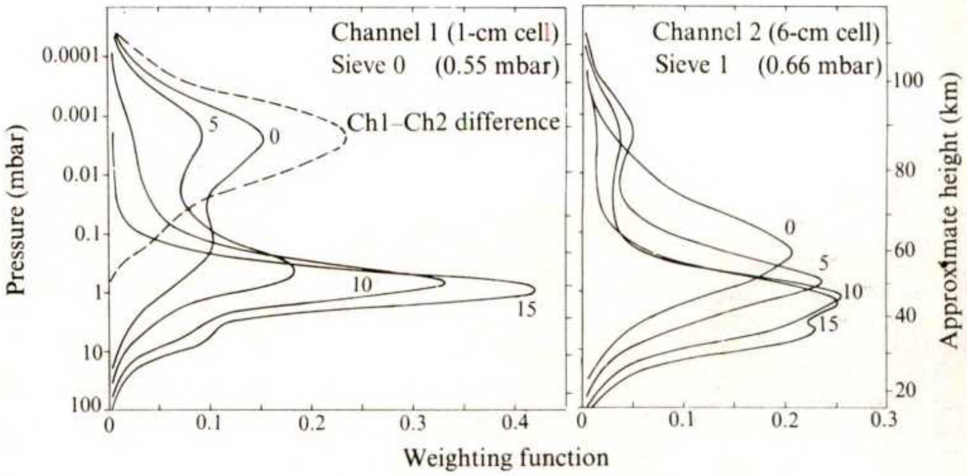
The Nimbus 6 PMR has two modulators called channels 1 and 2 which have cells of path length 1 and 6 cm respectively. Two different methods are used to vary the altitude range being sounded. The first is to vary the mean pressure in a modulator cell between 0.5 mbar and 4 mbar by altering the temperature of some molecular-sieve contained in a thimble attached to the cell (and hence the amount of gas adsorbed on it).

The second method uses Doppler scanning. Observing at a few degrees from the nadir along the direction of flight causes a Doppler shift of the incident radiation because of the velocity of the satellite. The CO_2 emission lines from the atmosphere are moved relative to the CO_2 absorption lines in the cell by an amount comparable with the width of the spectral lines in the mesosphere. With this spectral shift the pressure modulators select from narrow spectral intervals in the wings of the lines which originate from lower altitudes. Figure 1 shows a selection of weighting functions (the functions which describe the region of the atmosphere from which the radiation detected by the PMR originates) for different cell pressures and angles of view, showing that the altitude range 40–90 km can be well covered by such a combination. The field of view of the radiometer is 4° (full angle) along the direction of flight and 20° perpendicular to the flight direction, corresponding to a rectangle in the atmosphere of horizontal dimensions 70×350 km. Doppler scanning is arranged in flight by tilting the primary mirror of the optical system so that the direction of view moves from +15° to −15° with respect to the nadir along the direction of flight. One scan takes 88 s, the rate of scan being arranged so that during the scan the same part of atmosphere is being viewed.

The PMR has functioned continuously since June 20, 1975. From each day's data, hemispheric maps have been drawn for each of the channels of the radiance at the centre and end of the scan respectivey. Examples are shown in Fig. 2 for the Southern Hemisphere, for August 25, 1975, a day on which there was considerable planetary wave activity. For the lowest level shown (~45 km) notice the warm region at ~90° longitude and 65°S latitude. The wave amplitude is higher at ~ 50 km and the warm region has shifted westward to ~ 15° longitude. The westward shift continues with increasing altitudes to ~ 65 km but the wave amplitude decreases. Notice that the wave can still be identified at the highest level (~ 85 km altitude). A westward tilt of geopotential height is well known[6] to be associated with waves in which the energy is propagating upwards. A considerable amplitude of wave number two (two maxima round the pole) is evident at the lower levels, but has not penetrated far into the mesosphere.

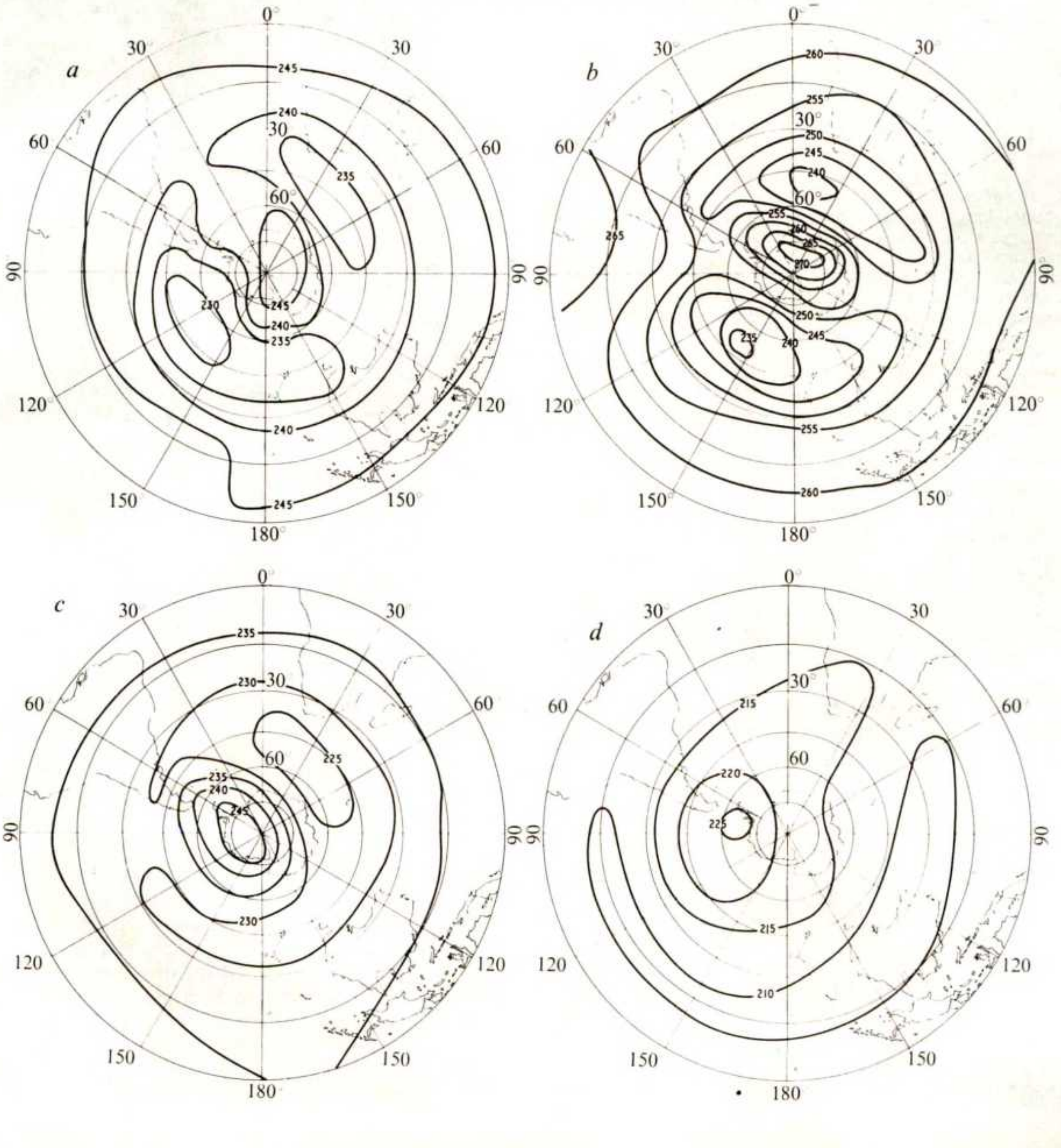
The radiance field of the top PMR channel (Fig. 2*d*) seems at first sight to be surprisingly flat, and does not

Fig. 1 Weighting functions for the 2 channels of the PMR for their lowest cell pressures for different angles of view from the nadir. The dashed weighting function in *a* is the weighting function applicable to the nadir view for 1.67 × (channel 1) − 0.67 × (channel 2).



indicate the presence of large amplitude gravity waves often observed by meteorological rockets. This is because the vertical wavelengths of such disturbances are short compared with the vertical averaging of ~ 20 km produced by the PMR. It is likely also that they possess horizontal distance scales which are short compared with the size of the field of view. The day-to-day consistency of the radiance fields at the top level demonstrates that there is little

Fig. 2 Polar stereographic maps of the Southern Hemisphere radiance (expressed as equivalent temperature) for August 24, 1975 for *a*, channel 2, 0.66 mbar, 15° from nadir; *b*, channel 1, 0.55 mbar, 15° from nadir; *c*, channel 2, 0.66 mbar, nadir view; *d*, 1.67 × (channel 1) − 0.67 × (channel 2) nadir view, cell pressures as in *a*–*c*. The appropriate weighting functions are shown in Fig. 1; they peak at ~40 km (*a*), 48 km (*b*), 61 km (*c*), 87 km (*d*).



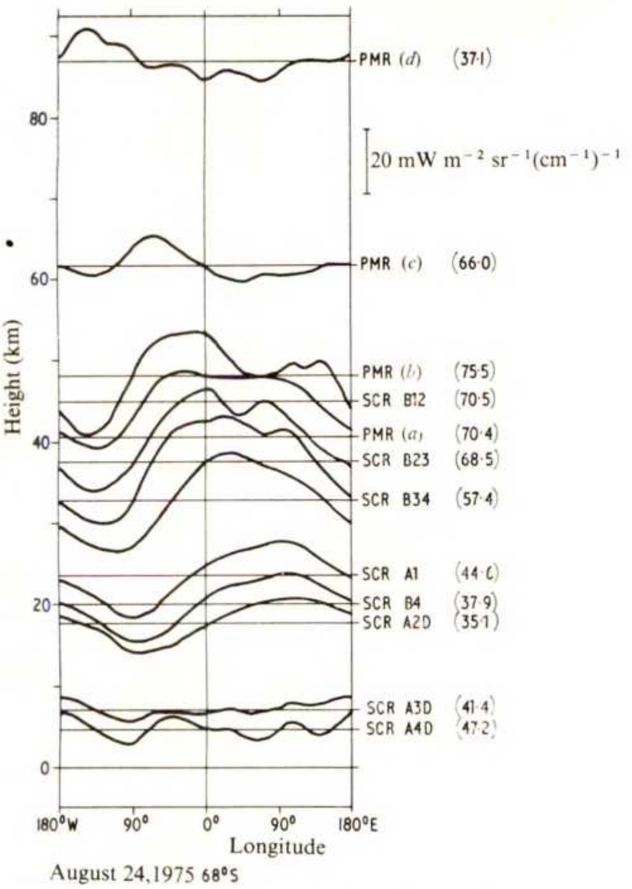


Fig. 3 Height–longitude cross section around the 68° S latitude circle for August 24, 1975. For each PMR and SCR channel the mean radiance is shown in brackets near the centre of the weighting function peak, and the deviation from the mean radiance is plotted as the ordinate at each of the levels. The PMR channels are labelled (*a*), (*b*), (*c*) and (*d*) corresponding to the key of Fig. 2.

residual atmospheric noise left after this averaging process.

Figure 3 gives more details of the wave. The PMR and Nimbus 5 SCR (which has functioned continuously for 3 yr) are in excellent agreement in the region where they overlap. The same planetary wave can be identified from the troposphere up to the mesopause, over which altitude range it shows a consistent westward tilt with altitude—the longitude of the maximum changing by nearly 360° from the bottom to the top.

Construction of the PMR involved a large number of personnel at Oxford University, the Rutherford Laboratory, Marconi's Space and Weapons Laboratory at Frimley, and the Department of Applied Physics, Reading University. The project was supported by the SRC. We also acknowledge the cooperation of the Nimbus Project, NASA.

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Received December 4, 1975; accepted March 16, 1976.

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Ball lightning

A DRAMATIC and characteristic ball lightning event, important because it may reveal details of the energetics of the phenomenon, was reported by a housewife to have occurred during a vigorous thunderstorm in the Midlands area of England on August 8, 1975. During this storm, a number of houses in the area were struck by lightning.

The witness was in the kitchen of her house in Smethwick, Warley, at about 1945, when a sphere of light appeared over the cooker. The ball was ~ 10 cm across and surrounded by a flame-coloured halo; its colour was bright blue to purple. The ball moved straight towards the witness at an estimated height of 95 cm from the ground. Burning heat was felt, and there was a singeing smell. A sound something like a rattle was heard. The ball was in sight for only a second or so because its lifetime was cut short:

"The ball seemed to hit me below the belt, as it were, and I automatically brushed it from me and it just disappeared. Where I brushed it away there appeared a redness and swelling on my left hand. It seemed as if my gold wedding ring was burning into my finger."

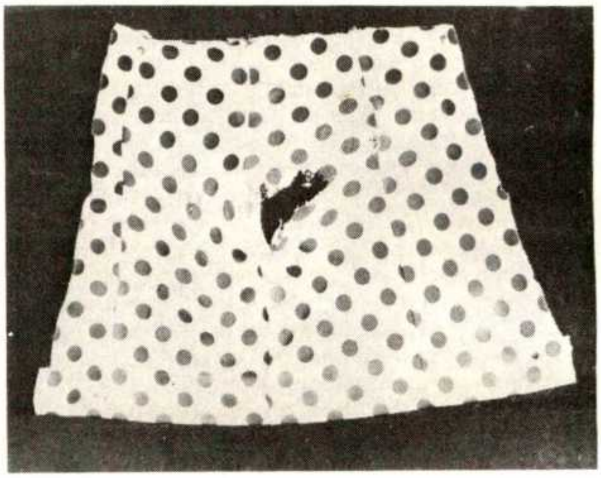

Fig. 1 Part of the dress alleged to have been damaged by "ball lightning", showing area and extent of damage. Scale: average diameter of spots in pattern is 19 mm.

Where the ball struck her, the clothing of the witness was damaged. A hole was produced in her dress and her tights. Her legs were not actually burned, however, but became red and numb. The dress has been secured for examination, and tests are currently being carried out to try to establish the nature of the damage. There is a central area (Fig. 1) where the synthetic fibre of the dress has disappeared altogether, and the shape of the hole is in agreement with the witness' statement that she brushed the ball downward a couple of inches. The hole is ~ 11×4 cm. Around its perimeter, the material has shrivelled, but is not charred. In addition, there is a secondary area where the fibre has shrivelled, and the pattern printed on the fabric has faded noticeably. The position of the primary damage is ~ 20 cm from the hem of the dress, so it is estimated that the ball struck at ~ 95 cm from the ground.

Although thunder and lightning were going on at the time, the witness is not sure whether or not the manifestation was associated with a thunderclap. The ball exploded with a bang, but she is not certain whether it exploded at the exact time at which she touched it; she felt this to be the case, however. Before my contacting her, the witness had had no acquaintance with the phenomenon of ball lightning.

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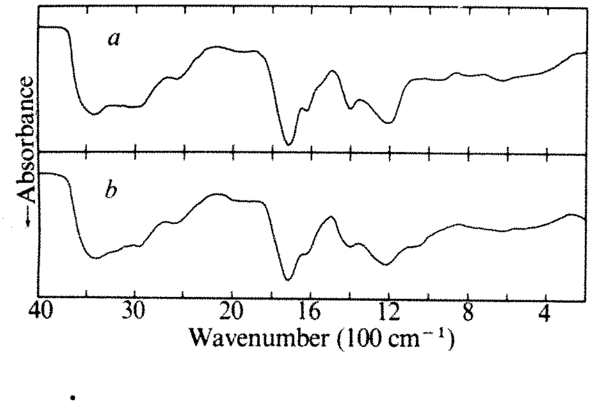
Possible relationship between soil fulvic acid and polymaleic acid

In the course of structural investigations into the organic matter component of soil, it became clear that the fulvic acid solutions[1] (the alkali-soluble fractions of humus not precipitated by acid) contained a high proportion of their soluble organic matter as a polymer, consisting of a carbon skeleton highly substituted by carboxyl groups. The solutions also normally contained peptide and polysaccharide mixtures or complexes which could be removed almost entirely by fractionation on charcoal[2], a technique which allowed the isolation of the polycarboxylic acid. Brown acidic polymers virtually identical with those found in the fulvic acid solution were recovered from podzol B_h horizon humus by extraction of the soil with dilute mineral acid or trisodium citrate solutions (*p*H 7.0) followed by dialysis.

Infrared spectroscopy, elemental analysis and a study of the degradation products of these polymers and their derivatives suggested that the polymers had an aliphatic or alicyclic backbone substituted predominantly by vicinal carboxyl groups. The soil polymers still contained polysaccharide, phenolic derivatives and nitrogen, but in low amounts, the last being accountable as amino acid residues and the ammonium ion. The product arising from the hydrolysis of pyridine-catalysed homopolymerised maleic anhydride[3] was found to be chemically and structurally related to the natural polymers, as shown by the similarities between their infrared absorption spectra (Fig. 1), their analysis (polymaleic acid: C 48.76, H 3.30, N 0.82%; B_h citrate-soluble humus: C 47.62, H 3.39, N 0.86%; for comparison, fulvic acid polymer: C 46.18, H 2.68, N 0.95%) and certain of their acid-hydrolysis products (Fig. 2). The dissimilar hydrolysis products, levulinic acid and the phenolic acids, could arise from species adsorbed on to or incorporated into the natural polymer, and this was demonstrated with the synthetic polymer. Slight differences between the potentiometric titration curve of the polymaleic acid[4] and that of the soil polymer were similarly explicable, while the solution-*p*H visible absorption spectra relationships were identical.

Fig. 1 Infrared spectra of *a*, polymaleic acid, *b*, fulvic acid in KBr pressed disks (1-mg sample and 170-mg KBr in a 12-mm diameter disk). Absorption in (*b*) near 1,000–1,100 cm⁻¹ is from carbohydrate.



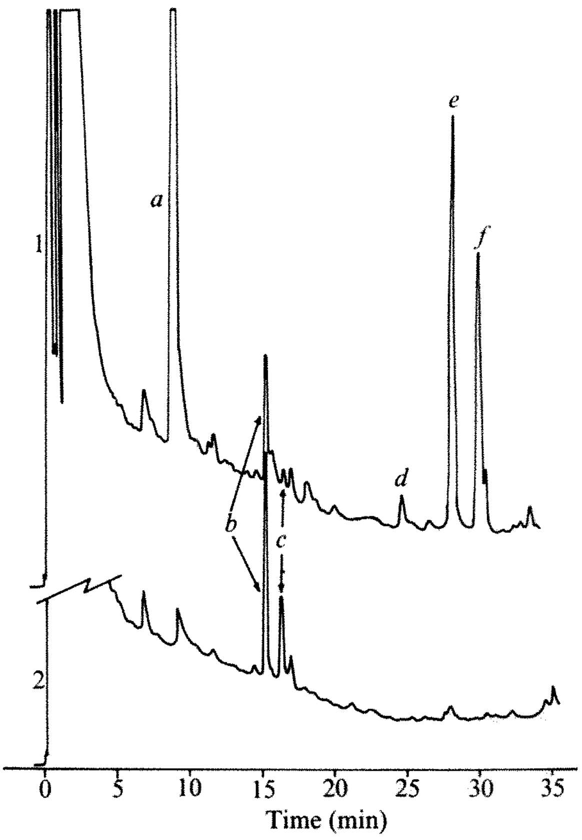


Fig. 2 Gas chromatography curves of trimethylsilyl derivatives of ether-soluble products from 6 M HCl hydrolysis of (1) Humus podzol Bh citrate-soluble humus, (2) Polymaleic acid. Products identified by gas chromatography–mass spectrometry: *a*, Levulinic acid TMS; *b*, Succinic acid di-TMS; *c*, Fumaric acid di-TMS; *d*, 4-Hydroxybenzoic acid di-TMS; *e*, Vanillic acid di-TMS; *f*, 3,4-Dihydroxybenzoic acid tri-TMS. GC conditions: 3% OV-1 on AW silanised Diatomite C (1.5 × 0.006 m); 15 cm³ min⁻¹ nitrogen carrier gas; temperature programme 100 °C isothermal for 5 min, 4 °C min⁻¹ to 250 °C, isothermal at 250 °C for 10 min.

Although the complete structure of polymaleic acid has yet to be elucidated, certain aspects of the structure are known, for example, the existence of vicinal carboxyl groups readily dehydrating to yield anhydride, a property shown spectroscopically by fulvic acid, and unsaturation which accounts for an absorption band near 1,620 cm⁻¹ in the infrared spectrum of fulvic acid. It is these features that make this polymer attractive as a model for fulvic acid. Structures already proposed[5] for fulvic acid are based on aromatic residues that have been assumed to arise partly from lignin- and/or tannin-derived material. Previous unpublished work by H.A.A. and A. Hepburn has shown that the yields of aromatic molecules from degradations are sufficiently low for these compounds to be considered of minor importance in the structure. Further structural investigations of fulvic and polymaleic acids are in progress.

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Received November 22, 1975; accepted March 11, 1976.

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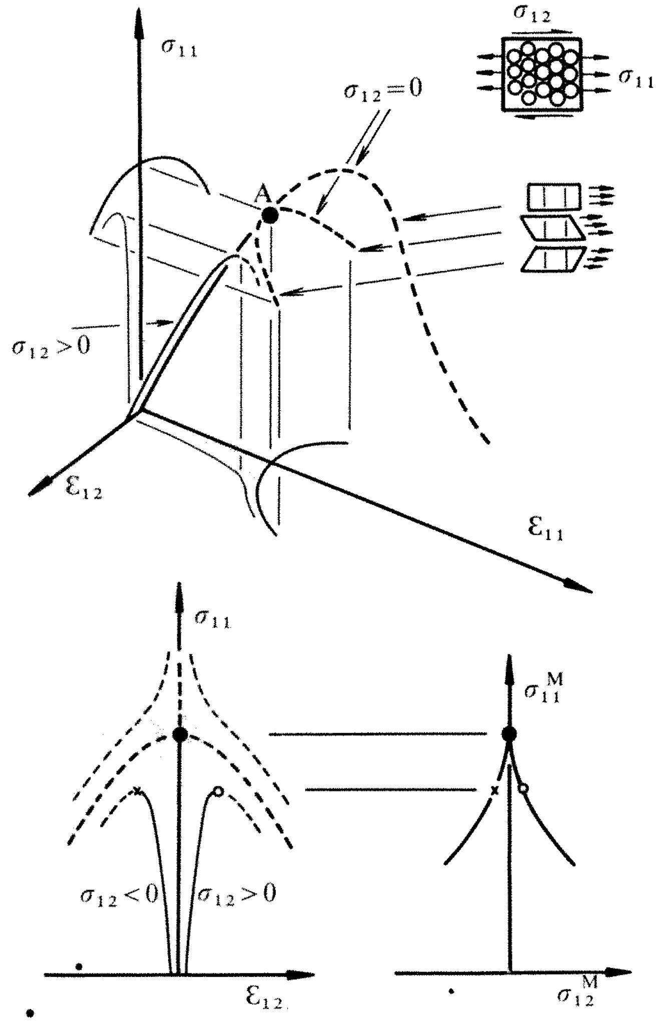
Hyperbolic umbilic catastrophe in crystal fracture

WE have demonstrated the existence of a cusp catastrophe in a symmetry-breaking mechanical instability of a close packed atomic lattice[1,2], and in an extension of this work we demonstrate here the existence of the more complex hyperbolic umbilic catastrophe[3-5] in a three-dimensional failure-stress locus.

The original cusp was associated with an unstable symmetric point of bifurcation[6] encountered by a close packed crystal with Lennard-Jones interatomic potentials subjected to a uniaxial tensile stress σ_{11}. Under this stress the lattice follows a primary equilibrium path involving a direct strain ε_{11}, and we showed that this path becomes unstable at the critical point A of Fig. 1. At this bifurcation point an unexpected symmetry-breaking shearing strain ε_{12} would develop explosively in the presence of even an infinitesimal disturbance, leading to a violent fracture of the crystal.

In this situation a corresponding small shearing stress σ_{12} acts as an 'imperfection' rounding off the bifurcation as shown by the lighter lines and giving rise to a sharp two-thirds power law cusp in the failure-stress locus represented by the plot of the peak stresses $\sigma_{11}{}^{M}$ and $\sigma_{12}{}^{M}$. This locus corresponds to the well known imperfection-sensitivity curves in the buckling of engineering structures[6-8].

Fig. 1 The upper picture shows the equilibrium paths of the atomic lattice when $\sigma_{22} = 0$, while the lower left-hand picture shows the projection of these paths into the (σ_{11}, ε_{12}) plane where we see the well known unstable-symmetric point of bifurcation or cusp catastrophe[6]. In general, stable regions of paths are drawn as solid lines while unstable regions of paths are drawn as broken lines. The lower right-hand picture shows the corresponding stability or catastrophe boundary as a failure-stress locus with the predicted two-thirds power law sensitivity.



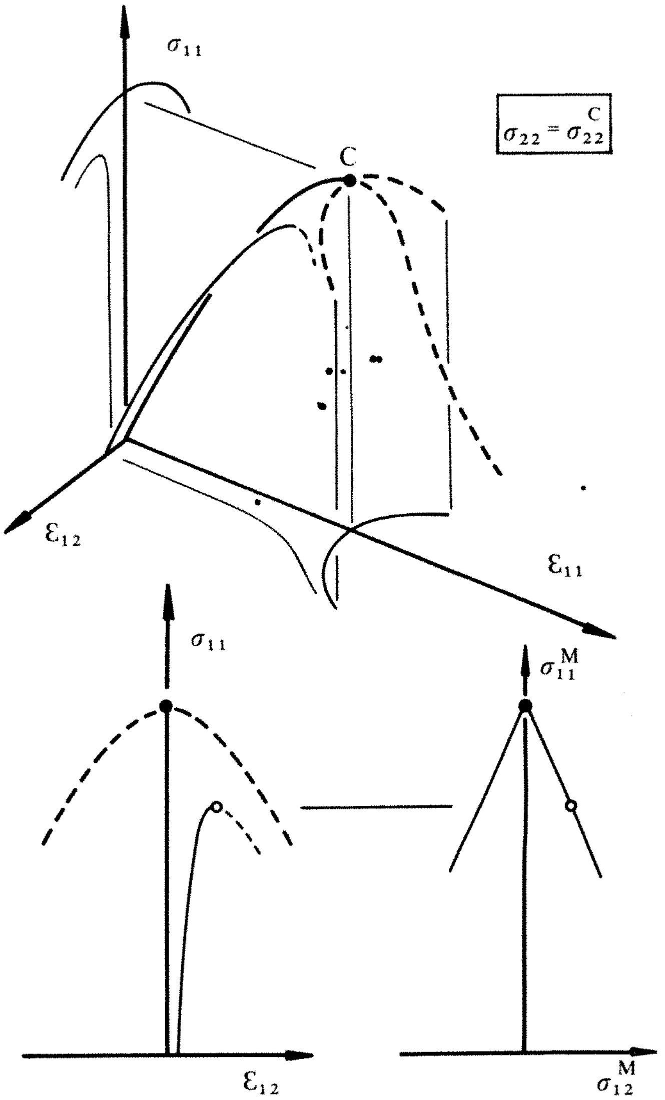


Fig. 2 This shows how Fig. 1 is modified by the addition of the direct stress $\sigma_{22} = \sigma_{22}{}^{C}$ which has shifted the point of bifurcation to the maximum of the primary stress–strain curve. The failure locus is now locally bilinear.

We have now extended this study by adding additionally a second direct tensile stress σ_{22} on the horizontal faces of the lattice. Within our approximations[2], which include an assumption that the vertical close packed columns undergo a negligible change in length, this direct stress has no effect on the primary equilibrium path lying in the plane of σ_{11} and ε_{11}. It does, however, influence the stability of this path, and has the effect of displacing the critical branching point A along the path. As we would expect, a negative (compressive) stress displaces A towards the unloaded state while a positive (tensile) stress has a stabilising action and displaces A in the direction of positive ε_{11}.

We have evaluated the magnitude of this stress, $\sigma_{22}{}^{C}$, that is necessary to shift the point A precisely to the maximum of the primary equilibrium path to give the situation shown in Fig. 2. In the presence of this small tensile stress $\sigma_{22}{}^{C}$ the crystal loaded by σ_{11} will thus encounter a hill-top branching point[2] at which the two-thirds power law cusp is replaced by a bilinear failure locus as shown in the plot of the peak stresses $\sigma_{11}{}^{M}$ and $\sigma_{12}{}^{M}$.

Inspecting our equations and the form of the equilibrium paths it is easy to see that this hill-top branching point is in fact

Thom's hyperbolic umbilic catastrophe[3–5], so that the full three dimensional failure locus in the space of the planar stresses $\sigma_{11}{}^M$, $\sigma_{12}{}^M$ and $\sigma_{22}{}^M$ must have the form of Fig. 3. Here we see that on the crest defined by $\sigma_{12}{}^M = 0$ the sharp cusp degenerates at the singularity corresponding to $\sigma_{22}{}^M = \sigma_{22}{}^C$ into two straight lines and thereafter the curve has a parabolic form. This crest is curved between A and C where it corresponds to failure at a branching point, but it is straight and parallel to the σ_{22} axis between C and B where failure is associated simply with the maximum of the primary equilibrium path: in this latter range the bifurcation has moved beyond this maximum and therefore no longer initiates the failure of the crystal. The failure surface has the characteristic form of the stability boundary of the hyperbolic umbilic catastrophe[9] which has often been likened to a breaking wave.

This attractive and concrete example of the hyperbolic umbilic catastrophe can now be added to the imperfection-sensitivity curve determined by G. W. Hunt[1,6] for the buckling of a stiffened panel as a second illustration of this catastrophe in nonlinear elasticity[10].

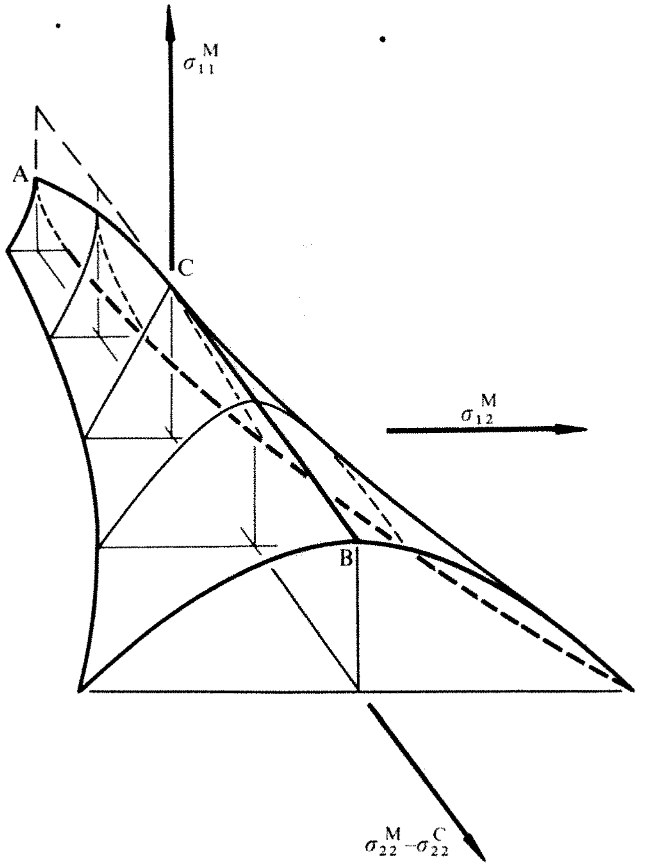


Fig. 3 The stability boundary in the space of the failure stresses corresponding to the catastrophe boundary of the hyperbolic umbilic catastrophe.

We feel that our demonstration of this new instability phenomenon for a fairly idealised, perfect and dislocation-free lattice is of some fundamental importance and is a useful complement to the numerical studies of Macmillan and Kelly[11] and the theoretical work of Hill[12]. Some further details of our cusp analysis have been published[13], and details of the present extension will be presented shortly.

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Received February 9; accepted March 8, 1976.

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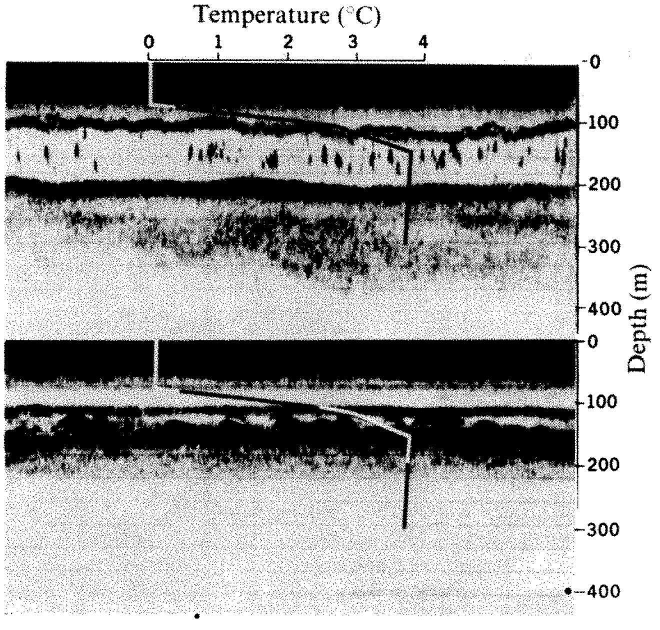
Arrest of deep scattering layer migration by a positive thermal gradient

LIGHT and temperature are considered to be the factors that influence diel vertical migrations of populations of biological sound scatterers (deep scattering layers) in the sea[1–3]. While light is probably the most important, temperatures which surpass the physiological tolerances of the organisms should play a major role in setting vertical limits. With positive thermal gradients (temperature increasing with depth) as are found in arctic and sub-arctic seas, sufficiently low temperatures near the surface could limit the evening ascent. This situation was observed in March, 1966 over the Kurile Trench along the eastern coast of the Kamchatka Peninsula (50°30′N, 162°30′E), an area influenced by the Oya Shio current system. Data were collected from USNS Charles Davis; acoustic records were made on a Westrex MK 10A Precision Depth Recorder using a Gifft transceiver to drive a hull-mounted UQN transducer at 12 kHz. The records were made on the 1.5-s sweep scale of the recorder.

Day and night acoustic scattering profiles are shown in Fig. 1. The day record was made between 1430 and 1530, March 5 while the ship was underway at 10 knots. The night record was made between 2330, March 5 and 0030, March 6 while the ship was drifting. Both records were made on the same station, however, and within a distance of ten nautical miles. The sea floor was at ~ 6,500 m. A 1,000-m hydrographic cast was taken at 2000, March 5 and

Fig. 1 Acoustic scattering profiles recorded over the Kurile Trench in March 1966. The upper record was made between 1430 and 1530 with the ship underway at 10 knots; the lower between 2330 and 0030 with the ship drifting. Temperature profile data from a hydrographic cast is superimposed.



the temperature structure is superimposed. Seven expendable bathythermographs taken within a ten-mile radius of the station show a similar profile. Salinity was ~ 33.2 ‰ from the surface to 100 m; at 200 m it had increased slightly to > 34 ‰.

The first well defined scattering feature below the outgoing pulse and surface scattering was a 20-m wide layer which oscillated between 90 and 130 m, the depth of the thermocline. The layer did not migrate, but seemed permanently associated with the thermocline on both day and night records. This phenomenon has been previously reported, and it has been demonstrated that the density gradient of the thermocline could not itself be the scattering factor for frequencies as high as 12 kHz (refs 5 and 6). Oscillations of the layer are suggestive of internal waves on the thermocline.

Between the thermocline layer and the main scattering layer were many discrete reflectors. Little is known of the composition of these targets over the deep sea, but their recordings are similar to acoustic signatures of fish schools in coastal waters[6]. The spatial relationship between these targets and the layers was maintained on both the day and night recordings. Like the main scattering layer beneath them, their upward migration was apparently arrested by the low temperature.

Maximum depth to the top of the main scattering layer was 255 m at 1000, March 5. Below the dense upper component of the layer, diffuse scattering was present to 350 m. Upward migration began at 1300 and a very gradual ascent (~ 20 m h^{-1}) progressed through the afternoon. The ascent was terminated at 1800 at a depth of 145 m.

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Received December 10, 1975; accepted March 8, 1976.

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A- and *B*-type hydrogen bond modifications of potassium hydrogen tartronate

ACCORDING to Speakman[1] two types of intermolecular hydrogen bonds between carboxyl groups can be discerned in the crystal structures of acid salts of carboxylic acids. The first, type *B*, occurs when two carboxyl groups are not symmetry related, and have structural differences sufficient to characterise them, separately, as unionised ($-CO_2H$) and ionised ($-CO_2^-$). Thus, any hydrogen bond between these groups must be asymmetric. The second, type *A*, occurs when two carboxyl groups are related by a symmetry element of the crystal, so that they are effectively equivalent. Thus any hydrogen bond between the groups must be, at least formally, symmetrical.

A reliable tool for establishing the type of hydrogen bond present in crystals of acid salts is infrared spectroscopy, which can be used to examine the region of the stretch vibration of the hydroxyl group[2]. Also, geometric evidence from X-ray data on a number of acid salts unambiguously reveals the existence of the two types of hydrogen bonds. The difference in symmetry brings about a significant difference in the geometry of the carboxyl groups involved in hydrogen bonding[1].

Moreover, there is also a difference in the O···O distance: in the *A* type it seems to be at least 0.05 Å shorter than the *B* type. It has been argued that the symmetry in the *A* type could be only apparent, and attributable to a 1:1 disordering of the hydrogen atom in an asymmetric hydrogen bond[3]. But it seems from infrared and X-ray studies that *A*- and *B*-type hydrogen bonds do differ. It is intriguing to identify the factors that determine whether a compound crystallises either as an *A*- or *B*-type salt. It is of interest to note that in the crystal of potassium hydrogen *meso*-tartrate both types of bond are present[4,5]. The *A* type relates to one *meso*-tartrate carboxyl group, and the *B* type relates to the second, which is inequivalent to the first because of molecular asymmetry.

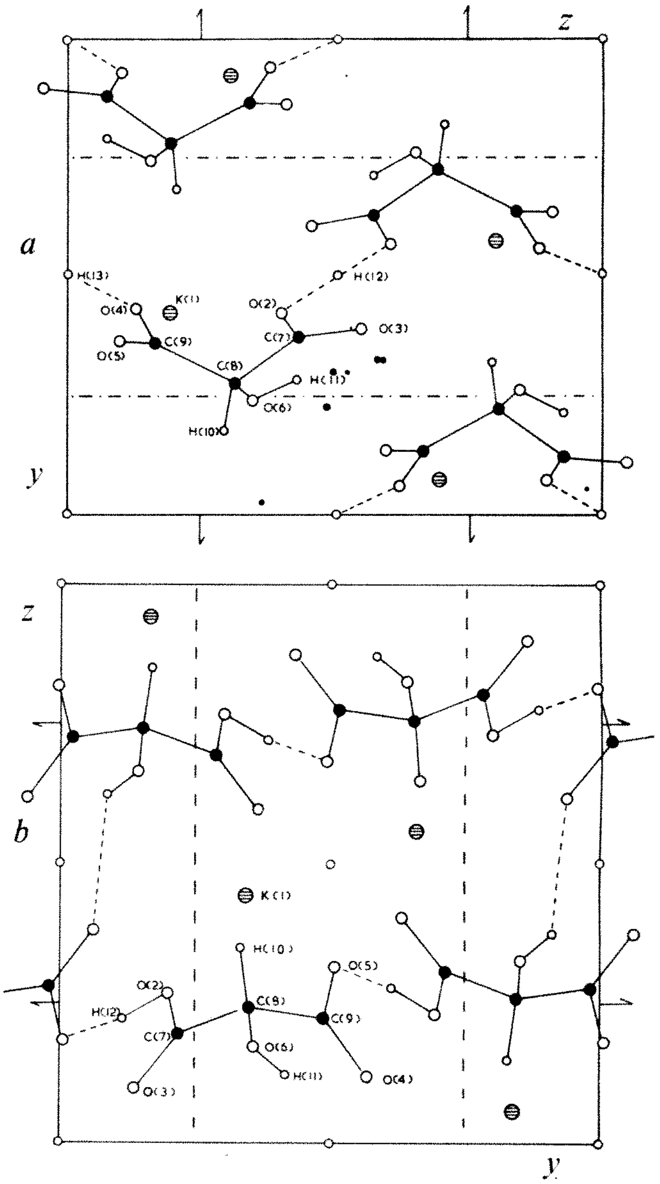

Fig. 1 Projections along the *a* axis of the crystal structures of potassium hydrogen tartronate: *a*, α form; *b*, β form. Dashed lines, hydrogen bonds.

The study of the *A*- and *B*-type hydrogen bonds found in the acid potassium salt of tartronic acid seems to be more promising in studies of the problem. That compound crystallises in two forms, the α form having an *A*-type and the β form having a *B*-type hydrogen bond. Experimental data of the modifications are summarised in Table 1, and relevant structural data are given in Table 2.

The *A*- and *B*-type hydrogen bonds are easily recognised from projections along the *a* axis (Fig. 1). The tartronic acid anions in both structures are linked by hydrogen bonds forming infinite chains. In the α form, the chains are generated by centres of inversion lying along the [101] direction with O···O

Table 1 X-ray data of the crystal structures of potassium hydrogen tartronate

From ethanol–water solution α form		From aqueous solution β form
Monoclinic, $P2_1/n$ $a = 7.356$; $b = 8.116$; $c = 9.197$ (Å) $\beta = 94.81°$, $V = 547.1$ Å^3 $D_x = 1.92$ g cm^{-3}; $Z = 4$		Monoclinic, $P2_1/c$ $a = 6.532$; $b = 9.248$; $c = 9.505$ (Å) $\beta = 99.74°$, $V = 565.8$ Å^3 $D_x = 1.86$ g cm^{-3}, $Z = 4$
1,129 independent reflections		1,204 independent reflections
	MoKα radiation, $\lambda = 0.7107$ Å	
	Absorption correction	
μ (MoKα) = 9.7 cm^{-1}		μ (MoKα) = 9.4 cm^{-1}
	Block-diagonal least-squares refinement K,O,C: positional and anisotropic thermal parameters. H: positional parameters, B fixed; extinction coefficient $\varepsilon = (e^2/mc^2)^2\lambda^2/V^2r^*$	
$\varepsilon = 29.10^{-5}$ cm^{-1} $R = 0.042$		$\varepsilon = 34.10^{-4}$ cm^{-1} $R = 0.042$

distances of 2.433 and 2.435 Å, respectively. In the β form the O···O distance in the hydrogen bond propagating along the screw axis is 2.485 Å. Glide planes interrelate the anion chains in both types. The aliphatic hydroxyl group interaction is very different in the two forms. In view of its geometry (Table 2), it is highly questionable whether the O-H···O contact in the α form should be considered as a hydrogen bond. In this respect we refer to the crystal structure of tartronic acid itself[6], in which the aliphatic hydroxyl group is free[7]: (O···O, 3.02 Å; H···O, 2.24 Å; O-H···O angle, 142°).

In contrast, the OH group in the β form participates in a hydrogen bond of normal geometry (Table 2). The molecular geometry and conformation in the anion part of the α form differs significantly from that of the β form. In the former the conformation of one of the carboxyl groups with respect to the C(α)-hydroxyl group is antiplanar, which is quite exceptional[8]. The observed geometry of the carboxyl group reflects the schematical classification in *A* and *B* types: the *A*-type carboxyl groups are half ionised, whereas in the *B*-type anion one carboxyl group is neutral and the other is completely ionic (Table 2). The potassium coordination is eightfold and, as usual, irregular.

From the shorter O···O distances in *A*-type hydrogen bonds it looks as though such a symmetrical bond is more favourable than an asymmetric one. We feel that the comparison of the crystal structures of the two modifications of potassium hydrogen tartronate provides new evidence for this. The energetically unfavourable molecular conformation and the weaker O(6)-H(11)···O(5′) hydrogen bond may compensate for the stronger *A*-type hydrogen bonding in the α form, and thus account for the dimorphism.

Table 2 Some geometric details of the carboxyl groups and hydrogen bonds in the α and β forms of potassium hydrogen tartronate*

	α form	Δ	β form	Δ
C(7)-O(2)	1.277(3)	0.054	1.302(4)	0.084
C(7)-O(3)	1.223(3)		1.218(4)	
C(9)-O(4)	1.281(3)	0.055	1.233(4)	0.044
C(9)-O(5)	1.226(3)		1.277(4)	
C(8)-C(7)-O(2)	114.6(2)	4.7	112.1(2)	11.7
C(8)-C(7)-O(3)	119.3(2)		123.8(3)	
C(8)-C(9)-O(4)	115.0(3)	5.5	118.7(3)	2.6
C(8)-C(9)-O(5)	120.5(2)		116.1(2)	
Hydrogen bond *DHA*	*D*···*A*	*D*···H	H···*A*	*D*···H···*A*
(α form)				
O(2)H(12)O(2′)	2.433(4)	1.216(2)	1.216(2)	180
O(4)H(13)O(4′)	2.435(4)	1.218(2)	1.218(2)	180
O(6)H(11)O(5′)	2.885(4)	0.85 (5)	2.42 (5)	114(3)
(β form)				
O(2)H(12)O(5′)	2.485(3)	1.06(5)	1.43(5)	173(3)
O(6)H(11)O(4′)	2.860(3)	0.92(3)	2.24(5)	152(3)

*Distances in Å; angles in degrees; e.s.d. in parentheses.

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Received December 31, 1975; accepted February 25, 1976.

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Speed of podzolisation

THE coastal sand dunes of eastern Australia are being mined extensively for heavy minerals. After mining, the replaced sand is subject to soil-forming processes. On such sites we have observed the formation of podzol profiles after 4.5 yr, with the development of bleached A_2 'pipes' up to 45 cm deep after 9 yr, indicating the extreme rapidity of soil-forming processes in suitable conditions.

Only a few estimates have been made of the time required for a podzol profile to develop because the time of initiation of soil-forming processes cannot usually be determined precisely. Chandler[1] used the known retreat pattern of the Medenhall glacier in Alaska and by comparing the degree of podzol development in materials of different ages, estimated that a mature profile would require 1,000–1,500 yr to form. Tamm (quoted by Russell[2]) used the drainage of Lake Ragunda in 1796 as the beginning of soil development and also estimated a time of from 1,000–1,500 yr for the development of a mature podzol. In Australia, Burges and Drover[3] examined soil development on a sequence of beach ridges just north of Sydney, and estimated that a period of 1,000 yr is required to develop a distinct B horizon in an iron podzol. The basis of this estimate was not the soil profile itself, but the supposed age of the beach ridges in which the podzols are developed.

On a more restricted time scale, Crompton[4] demonstrated that thin iron pan podzols, not more than 8 cm in total thickness have developed in less than a century. Muir[5]

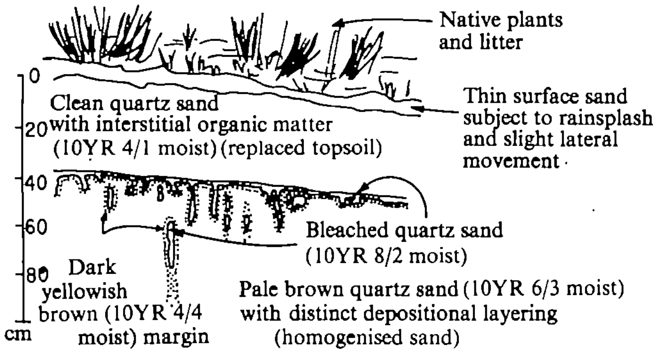
Native plants and litter
0
Clean quartz sand
20 with interstitial organic matter (10YR 4/1 moist) (replaced topsoil)
Thin surface sand subject to rainsplash and slight lateral movement
40
60
Bleached quartz sand (10YR 8/2 moist)
80
Dark yellowish brown (10YR 4/4 moist) margin
Pale brown quartz sand (10YR 6/3 moist) with distinct depositional layering (homogenised sand)
cm

Fig. 1 Soil profile in revegetated sand 9 yr after mining (sketch from photograph).

reported that on formerly cultivated land overgrown with Scots pine in Scotland a bleached A$_2$, 0.5 cm thick, developed in 20 yr.

The example we have studied is in the Myall Lakes area, 290 km north of Sydney (Portion 19, Parish of Fens, New South Wales, Private Mining Lease 2, 32°39′S 150°11′E). Here, before mining, well developed podzols supporting dry heath or woodland occurred in low relief quartz sand dunes. The heath vegetation was similar to that described by Clark[6] and included shrubs such as *Banksia, Leptospermum, Casuarina* and *Persoonia,* up to 3 m high. The original profile was: dark grey A$_1$ 0–30 cm; bleached A$_2$ 30–150 cm, sharply demarcated across a highly convoluted boundary from the B$_2$ which had a marked colour contrast but only slight and sporadic induration developed in several metres of sand.

The oldest site examined supported heath and was mined in September 1966. During this process the podzol was completely destroyed, since mining involves the stripping and stockpiling of the topsoil (largely A$_1$), and complete homogenisation of all remaining sand to depths greater than 5 m by the dredging and mineral separation processes. The pale brown (10 YR 6/3 moist 7/3 dry) homogenised sand was reformed to a similar topography as the original and covered by the stockpiled topsoil during March 1967. The area was planted with rye (*Secale cereale*), West Australian blue lupin (*Lupinus angustifolius*) and fertilised with Shirleys No. 17 fertiliser (9.2% N, 4.3% P, and 4.6% K) at the rate of 517 kg ha^{-1}, during September 1967. Aerial fertilisation with Nitram (34% N) at the rate of 129 kg ha^{-1} was carried out in April and September 1972. Regrowth of native species occurred from seed in the replaced topsoil. The present vegetation on the mined site is 0.5–1.5 m high, covers 50% of the ground surface (visual estimate) and is made up of shrubs which occur on adjacent unmined areas but in different proportions.

Figure 1 is a section through the sand 9 yr after mining. Below the abrupt lower boundary of the replaced topsoil, the underlying homogenised sand has a zone of bleaching with an extremely convolute lower margin marked by a thin zone of dark yellowish brown (10 YR 4/4 moist and dry) sand. On examination under a binocular microscope the surface organic-rich material (replaced A$_1$) is seen to consist of clean quartz sand grains with interstitial organic matter. The homogenised unbleached sand is made up of approximately 80% clean quartz grains and 20% of grains with reddish (probably ferric oxide) skins on their surface, giving the overall pale brown colour. Within the bleached zone the surfaces of the quartz grains are completely clean and there is no interstitial organic matter. Bleaching of the pale brown homogenised sand is associated with a reduction of the iron content of the sand from about 1,150 p.p.m. (w/w) to about 250 p.p.m. Iron was extracted from ashed samples in hot concentrated hydrochloric acid and concentrations were determined by atomic absorption spectrophotometry. Dissection of Kubiena tin samples shows the bleached zone to be planar immediately beneath the replaced topsoil, to average 1–2 cm in thickness and to extend up to 45 cm deep as scattered roughly circular pipes. On a microscale this is an exact replica of the convoluted relationship seen between the A$_1$ and B$_2$ horizons of the podzols in unmined sand. It would seem to result from percolating solutions removing surface coverings of ferric oxides from quartz sand grains and depositing them again within a short distance, that is, in a typical podzolising process. The rapidity of this process in producing features of the dimensions shown in Fig. 1, in 9 yr is probably due to the absence of finer grained materials to inhibit the throughflow of solutions or to inactivate the molecules responsible for the podzolisation. It is not certain whether the active organic molecules originate from the present vegetation or from organic matter in the replaced topsoil. The pipe-like areas of bleached sand were also found on mined sand under *Imperata cylindrica* and in an area of sparse vegetation on a former *Ammophila arenaria* nursery. They were not, however, present under this vegetation where the organic content of the replaced topsoil was low.

The speed with which the pale brown quartz sand can be bleached has been demonstrated in the laboratory. Aqueous extracts from fresh leaves of *Angophora costata* and *Eucalyptus gummifera* growing on a podzol site were prepared using the method of Bloomfield[7]. These species are common in woodland on podzols at Myall Lakes. The 5% extract was dripped at 1–2 cm^3 h^{-1} into the top of a glass tube (0.3 cm internal diameter) packed with the homogenised mined sand. Continuous flow produced visible bleaching to a depth of a least 1 cm in the first hour. This extended to the bottom of the column (25 cm) after about 7 h. With interrupted flow but without drying out of the column, a bleached layer 2 cm deep formed above a dark brown band 0.1 cm thick within 18 h. Water or acetate buffer (*p*H 4) did not duplicate these effects. These experiments reproduce within hours aspects of the podzol morphology. They are being continued in an effort to determine the conditions for iron redeposition and the relative importance of vegetation species and soil organic matter in the genesis of these profiles.

Two points requiring evaluation arise from these observations; pedological and geomorphic ages based on the degree of development of podzol profiles, especially in sand dune areas, may have been overestimated and the speed of pedological processes, especially in disturbed systems, may have been underestimated.

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Received December 17, 1975; accepted February 6, 1976.

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Transfer of mildew resistance from the wild oat *Avena barbata* into the cultivated oat

MILDEW (*Erysiphe graminis*, F.sp. *avenae*) can reduce the yield of cultivated oats by 30% (ref. 1) and is the most important disease of the crop in the UK. The ability of the pathogen to change either by mutation or recombination, giving rise to strains which can effectively attack previously resistant cultivars, leads to a continuous search for further sources of resistance. Genotypes of related weed species of *Avena* have been isolated that are completely resistant to mildew. A genotype of the tetraploid species *Avena barbata* ($2n = 28$) collected in Algeria is resistant to all the prevalent races of mildew and would form a valuable source of resistance to the pathogen. We now report the successful transfer of this source of mildew resistance into the cultivated oat. This provides the oat breeder with a new source of mildew resistance to use in breeding programmes.

The cultivated oat *Avena sativa* is a hexaploid species ($2n = 42$) and probably combines three distinct diploid sets of chromosomes. *A. barbata* can be crossed readily with the cultivated oat, but the hybrid is sterile. It is possible to obtain the occasional viable seed when the sterile hybrid is backcrossed to *A. sativa* and some success has been achieved in transferring genes for crown rust resistance from diploid and tetraploid weed species to *A. sativa* by continuous backcrossing[2,3]. But the genetic control of chromosome pairing in *A. sativa*[4,5], which restricts pairing to homologous chromosomes, minimises the amount of interspecific chromosome pairing that takes place in hybrids and derivatives. Consequently, plants resistant to crown rust isolated in later generations of interspecific hybrids have often proved to be chromosome substitution or addition lines, and not the products of natural recombinations[6–8].

The amphiploid *A. barbata* × *A. sativa* was produced at the Welsh Plant Breeding Station and backcrossed twice to *A. sativa*. Selection for mildew resistance was practised at each generation and a resistant plant with 44 chromosomes was isolated from the selfed progeny of the second backcross generation[9]. This disomic addition line combines the full complement of the recurrent *A. sativa* parent Manod and a pair of *A. barbata* chromosomes. The monosomic addition line ($2n = 43$) is also resistant. When the selfed progenies of the monosomic addition line were tested for mildew reaction to race 2 all the resistant plants were found to contain the *A. barbata* chromosome, confirming that resistance is due to the presence of the *A. barbata* chromosome. No recombinants were found in the progeny (5,649 seedlings tested) of the monosomic addition line.

Seeds of the disomic addition line ($2n = 44$) were irradiated at doses of 10,000 and 15,000 rad using a ^{60}Co source. The screening procedure for translocations was that used successfully in wheat-rye studies[10]. If translocations between the *A. barbata* and *A. sativa* chromosomes were achieved, the formation of a multivalent or incompletely homologous bivalents would give rise to gametes deficient for the *A. barbata* gene for resistance. When the I_1 plants (originating from the irradiated seeds) are selfed fusion of gametes which do not have the resistant gene should result in some susceptible progeny in the I_2 populations. Nineteen I_2 families out of 391 did segregate a proportion of susceptible progeny but the rest produced only resistant seedlings, as expected from a normal disomic addition line. The resistant plants from the 19 I_2 families were grown to maturity to produce I_3 seeds. As a result of further tests for mildew reaction in the I_3 and I_4 generations, lines were isolated which segregated three resistant: one susceptible seedlings. All these segregating lines had 44 chromosomes, which shows that the susceptible seedlings were not the result of the complete loss of the *A. barbata* chromosome.

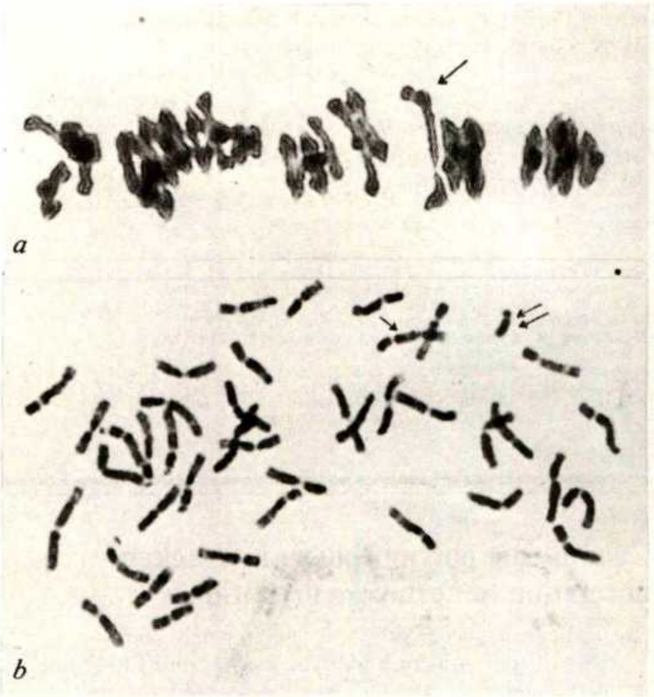

Fig. 1 *a*, Metaphase 1 in I_3 plant of B.133 with 22 bivalents, one of which is a distinct heteromorphic bivalent. *b*, Somatic chromosomes of a euploid plant heterozygous for the translocated chromosome. Two arrows indicate natural chromosome 21 and a single arrow the translocated chromosome.

One of these particular lines, B.133, was studied in detail to assess the potential of the translocation stock as breeding material. Meiotic analysis was made difficult by clumping and stickiness of chromosomes, but in some cells a distinct heteromorphic bivalent (Fig. 1*a*) was observed. Resistant plants were crossed with the susceptible cultivar Manod to produce 43-chromosome hybrids. The F_2 families again segregated in a 3 : 1 ratio, the pooled data being 361 resistant : 120 susceptible seedlings. Counting the chromosomes of the resistant seedlings revealed that with the exception of the occasional seedling with 43 chromosomes the resistant progeny had the euploid number of 42 chromosomes. The *A. barbata* gene had been incorporated into the *A. sativa* genome and behaved normally, producing a monofactorial ratio.

Some of the chromosomes of *A. sativa* can be identified readily according to shape and size[11]. Chromosome 21, the smallest of the complement, can be identified easily. In the F_2 families which segregate three resistant : one susceptible we found that in the resistant seedlings chromosome 21 was either absent or only present as a single chromosome. In the susceptible seedlings the pair of chromosomes 21 was always present. Because all the seedlings had the euploid number of 42 chromosomes the fact that chromosome 21 was either absent or present single in the resistant seedlings shows that it is involved in the translocation. Resistant seedlings lacking chromosome 21 are homozygous for the translocated chromosome and those containing only a single chromosome 21 are heterozygous for the translocated chromosome. It has already been established that the gene for mildew resistance is located on the short arm of the *A. barbata* chromosome[9]. The morphology of the translocated chromosome (Fig. 1*b*) suggests that it involves the short arm, the centromere and a portion of the long arm of the *A. barbata* chromosome and the long arm of chromosome 21 of *A. sativa*.

Plants homozygous for the translocation are vigorous and fertile, the deletion of a part of chromosome 21 and the duplication arising from the *A. barbata* segment are readily tolerated. In the euploid lines which were heterozygous for the translocation 21 bivalents were regularly formed, one of which was heteromorphic. The regular monofactorial segregation observed when the heterozygous translocation stock is selfed shows that the translocated chromosome can compete effectively with the intact chromosome 21. It should thus be possible to transfer the mildew resistance to other cultivars of the cultivated oat by a simple backcrossing programme.

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Received January 19; accepted February 25, 1976.

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Cyanogenic polymorphism in bracken in relation to herbivore predation

THE ecological success of bracken is partly a result of its extensive rhizome system, and because of its ability to synthesise various secondary compounds which deter predators and phytopathogens[1–3]. These compounds include the cyanogenic glycoside, prunasin[4], toxic because on enzymatic hydrolysis HCN is released. Although present in only 5% of ferns[5], cyanogenic glycosides are ecologically significant in other plant species; they deter both molluscs and voles from grazing on *Lotus corniculatus*[6], and rabbits from eating *Trifolium repens*[7]. As in these two species, we have found that cyanogenesis is also polymorphic in bracken.

Our aim was to correlate herbivore predation with changes in cyanogenesis and phenolic content of bracken throughout the growing season (G.A.C.-D., E. Bernays, S. Finch and T.S., unpublished). We examined bracken from several randomly chosen sites in Richmond Park, Surrey, These comprised open and shady areas, and involved both inter- and intra-population studies. Our results showed that in most populations 96% of individuals contained both prunasin and the corresponding hydrolase and were therefore cyanogenic, but in a few populations, 98% of individuals were acyanogenic. Of these, 85% lacked both enzyme and substrate, whereas 15% contained prunasin without enzyme. We found that the bracken fronds from such areas were heavily grazed by deer and sheep, as examination of animal droppings in the area and observations by the gamekeeper showed. Examination of other widely separated patches of bracken confirmed that only fronds from acyanogenic plants were eaten.

To establish whether it is the production of HCN or merely the possession of prunasin which is the predator deterrent, we fed disks of bracken fronds to the phytophagous locust, *Schistocerca gregaria*. The results of our experiments showed that when both prunasin and enzyme were present only 4% of fronds were eaten, whereas when only the substrate was present the insects consumed 52% of the test meal, which was nearly the same as when both substrate and enzyme were absent (48%). It seems, therefore, that the production of HCN (or benzaldehyde) acts as the deterrent. Young fronds of bracken were more cyanogenic than older fronds, whether sampled throughout the season, or as young and spore-bearing fronds of a single plant. We found that *S. gregaria* consumed the minimum amount of bracken in test meals during late May to early June, coinciding with the greatest intensity of cyanogenesis[8].

We conclude that cyanogenic polymorphism in bracken has a positive role in determining the degree of herbivore predation and that there is selectivity for acyanogenic forms in natural conditions.

We thank Dr E. Bernays, C.O.P.R., Mr S. Finch, Brunel University, and Mr Brown, gamekeeper of Richmond Park, for their assistance. This work was supported by the ARC.

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Received January 21; accepted February 26, 1976.

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Inhibitory binocular interaction in human vision and a possible mechanism subserving stereoscopic fusion

IN the human visual system, pathways from the two eyes converge anatomically by partial crossing over of the optic nerve fibres at the optic chiasma. Neurophysiological studies of the primary visual cortex of primates have established that these converging pathways carry signals from both eyes to the same cortical neurones[1]. In normal viewing conditions, each eye forms an image of the external visual field, the point of attention in the field being centrally fixated in both eyes. There is a small difference in the relative positions of non-central image points formed on the two retinae because the eyes view the visual field from slightly different positions. The ability of the primate visual system to extract information about the depth of a focused object in the visual field apparently rests on the convergence on to single cortical neurones of signals arising from the slightly displaced images in the two eyes[2]. If each eye perceives a totally different visual stimulus, however, the visual signals arising from the two stimuli are perceptually antagonistic and, in this situation, an observer usually detects only one stimulus at a time. This phenomenon is referred to as binocular rivalry. We have investigated interactions between visual signals produced by two different stimuli when presented one to each eye.

Our studies concerned a special class of stimulus patterns—one-dimensionally periodic distributions of light which, because of their alternating light and dark bar structure, are called grating stimuli (Fig. 1 inset). The visual detectability of such grating stimuli is frequently specified by the minimum (or threshold) value of contrast level at which the grating can just be detected, with the contrast defined as $(I_{max}-I_{min})/(I_{max}+I_{min})$, I_{max} and I_{min} being respectively the maximum and minimum illumination levels present in the grating stimulus pattern. It has been shown that the threshold contrast level for detection of a grating increases transiently after adaptation to a similar grating of high contrast[3–5]. This adaptation effect is observed only when the test and adaptation gratings are of similar spatial frequency (that is periodicity) and when they are oriented in near parallel directions. The adaptation effect can be studied by presenting both test and adaptation gratings to the same eye[5], or by presenting them one to each eye[6], and both methods yield essentially the same results. We measured the grating adaptation effect with the test and adaptation gratings, matched in orientation and spatial frequency, presented to one eye and a further grating stimulus, which we call the conditioning grating, presented to the other eye (Fig. 1 inset). The results show that when the conditioning grating is of spatial frequency, f_{cg}, in the approximate range 1–4 cycles per degree, it suppresses the adaptation for all spatial frequencies, f_{ag}, of the test and adaptation gratings, except when f_{ag} is equal to f_{cg}. With the observer's head in the normal upright position, this suppression effect is observed only when all the gratings are orientated in a vertical direction and only when the conditioning and adaptation gratings are presented to different eyes.

The experimental arrangement of the visual fields is illustrated in Fig. 1 (inset). The grating patterns were produced by laser interference methods[6,7] and were in all cases sinusoidally modulated grating patterns of 100% contrast. The adaptation effect was measured in terms of Δ, equal to $\log I - \log I^1$, where I is the mean threshold illumination level of the 100% modulated test grating required for its detection after adaptation to the adaptation grating and I^1 the corresponding illumination level following adaptation to a uniform field. The illumination level of the uniform field and the mean illumination level of the adaptation grating were set equal at 4.5 log trolands, thus ensuring that both stimuli produced the same mean retinal adaptation condition. At the start of each measurement, the observer viewed the adaptation grating for 4 min and was instructed to scan the field during the periods of adaptation to ensure uniform retinal adaptation. As the grating adaptation effect is of short duration[5], the test and adaptation gratings were then alternated for periods of 3 and 20 s, respectively, the cycle being repeated until the observer obtained the threshold illumination level, I, for detection of the test grating. A similar procedure, in which the adaptation grating was replaced by a uniform field of the same mean illumination level, was used to determine I^1. The conditioning grating was presented in coincidence with the adaptation grating (Fig. 1 inset) and the observer's head was fixed by means of a dental clamp with the interocular direction horizontal. Data were obtained for three observers, all of whom gave substantially similar results, and the data presented here refer to E.W. who made the most extensive observations.

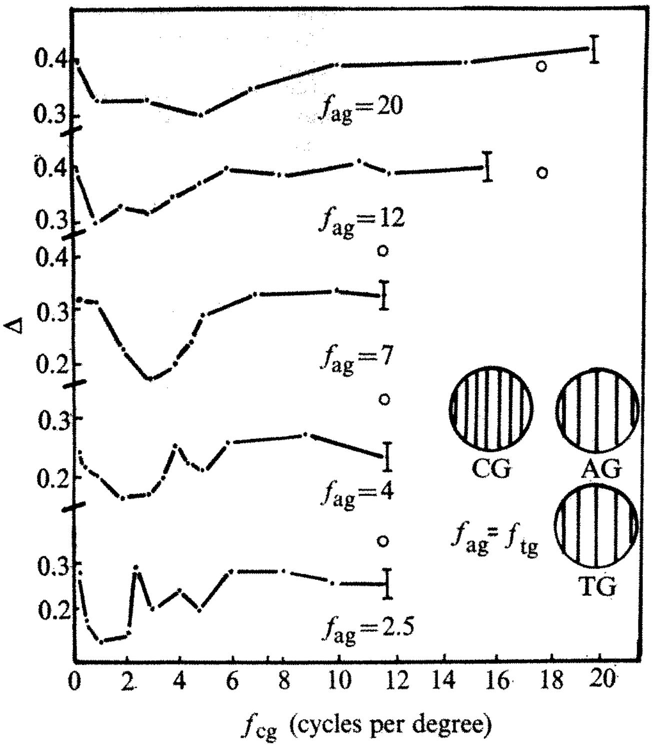

Fig. 1 Values of Δ plotted against the spatial frequency, f_{cg}, in cycles per degree of the conditioning grating. Plots are given for five spatial frequencies, f_{ag}, of the test and adaptation gratings and the values of f_{ag} corresponding to each plot are given in cycles per degree to the right of the data points. Experimental points, denoted by the full circles, are the mean of 10 observations, and typical standard errors are denoted by the vertical error bar given with the highest frequency point of each plot. All other standard error values were similar in amplitude to those defined by the bars. For each value of f_{ag}, Δ was also determined in the absence of the conditioning grating, and the values obtained in this way are plotted as open circles to the right of each plot. All grating stimuli were of wavelength 632.8 nm and the illumination level of the adaptation and conditioning gratings was 4.5 log trolands throughout. All gratings were orientated in a vertical direction. The inset shows the appearance of the visual fields in the two eyes. During periods of adaptation, the conditioning grating, CG, was presented to the left eye and the adaptation grating, AG, to the right eye. The test grating, TG, was presented in isolation to the right eye. All gratings fields were 30°.

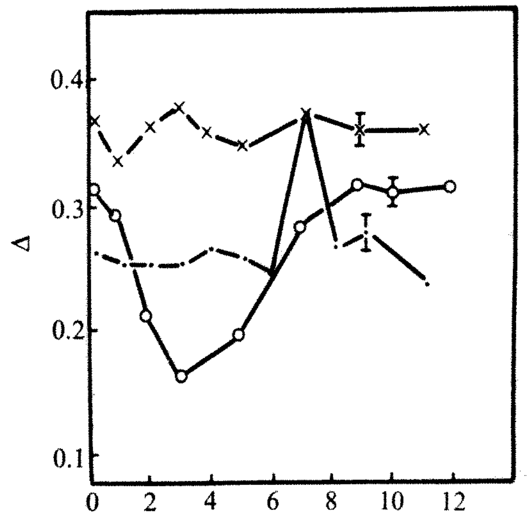

Fig. 2 Values of Δ plotted against the spatial frequency of the conditioning grating, f_{cg}, in cycles per degree. All values refer to test and adaptation gratings of spatial frequency 7.0 cycles per degree. Open circles refer to the stimulus configuration shown in Fig. 1, that is, with all gratings orientated vertically, the adaptation and conditioning gratings presented simultaneously to the right and left eyes, respectively, and the test grating presented to the right eye. Crosses refer to a similar stimulus configuration, but with all gratings oriented in a horizontal direction. Full circles refer to the arrangement in which the adaptation and conditioning gratings were both presented to the same eye as the test grating. All data points are the mean of 10 observations and typical standard error values are denoted by the vertical error bars. All stimuli were of wavelength 632.8 nm and the illumination level of the adaptation and conditioning gratings was 4.5 log trolands. $f_{ag} = f_{tg}$ = 7 cycles per degree.

The results obtained with the grating bars orientated in a vertical direction are given in Fig. 1, values of Δ being plotted against f_{cg}, the spatial frequency of the conditioning grating. Data are given for a number of values of f_{ag}, the common spatial frequency of the test and adaptation gratings, and the values of Δ obtained in the absence of the conditioning field are also given for each value of f_{ag}. These data show that there is a small decrease in Δ, relative to the value obtained in the absence of the conditioning stimulus, over much of the range of f_{cg}. For values of f_{cg} in the approximate range 1–4 cycles per degree, however, there is a large decrease in Δ, implying that the adaptation effect is suppressed in the presence of the conditioning grating. An exception to this is observed when the conditioning and adaptation gratings have the same spatial frequency, for example, at f_{cg} equal to 2.5 cycles per degree for the data referring to f_{ag} equal to 2.5 cycles per degree. When the experiment was repeated with the gratings oriented horizontally, however, the data showed that for all values of f_{cg}, except f_{cg} equal to f_{ag}, Δ is reduced by about 0.1 relative to its value in the absence of the conditioning grating (full circles in Fig. 2). Measurements with the gratings set at various angles between vertical and horizontal established that the grating adaptation effect is suppressed only for vertical and near-vertical orientations of the grating lines (Fig. 3). Further experiments were performed in which the adaptation and conditioning grating were both presented to the same eye as the test grating, all three gratings being orientated vertically. A typical set of data is given in Fig. 2 (crosses), values of Δ being plotted against f_{cg}, the spatial frequency of the conditioning stimuli. The large reduction in Δ for f_{cg} in the range 1–4 cycles per degree found when the adaptation and conditioning gratings are presented to different eyes, does not occur when they are presented to the same eye. Thus the suppression of the grating adaptation effect by a low frequency conditioning grating is specifically associated with binocular interaction. No low frequency inhibition of the kind seen in Fig. 1 is found when the adaptation grating is presented to one eye and the test grating to the other[6]. The inhibition of the grating adaptation effect associated with the conditioning grating seems, therefore, to arise from an interaction between the suprathreshold adaptation and conditioning gratings during their simultaneous presentation to different eyes.

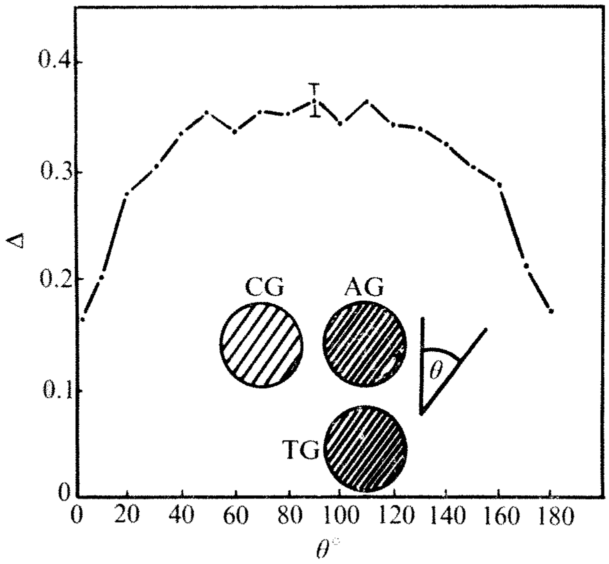

Fig. 3 Values of Δ plotted against θ, the angle at which the gratings were orientated from the vertical. The test and adaptation gratings were of spatial frequency 7.0 cycles per degree and the conditioning grating was of spatial frequency 3.0 cycles per degree. All points are the mean of 10 observations and the vertical error bars denote typical mean errors. The stimulus wavelength was 632.8 nm and the illumination level of the adaptation and conditioning gratings was 4.5 log trolands. f_{cg} = 3 cycles per degree; f_{tg} = f_{ag} = 7 cycles per degree.

The restriction of the binocular suppression effect to gratings orientated in a vertical direction suggests that it is involved in the stereoscopic fusion mechanism. Suppression of signals from all but matched low frequency components of images presented to the two eyes would facilitate stereoscopic fusion of low frequency elements of the visual field. Investigation of disparity-sensitive neurones in cat and monkey has revealed response features consistent with the characteristics of the binocular interaction described here. Thus, in the monkey, the long axis of the bar-shaped receptive fields of disparity neurones are preferentially orientated vertically[2], and in the cat, binocular inhibitory interaction is apparent in the responses of such neurones[8]. Stereoscopic fusion effects in human vision associated with periodic structures can be demonstrated more readily for low frequency than for higher frequency structures[9,10]. The spatial frequency range of inhibitory binocular interaction found in our experiments corresponds approximately to the range of preferred spatial frequencies revealed in these studies on stereoscopic fusion of periodic structures. We have considered the possibility that the observed binocular suppression of the grating adaptation effect is a result of binocular rivalry in perception of the adaptation and conditioning grating stimuli. Neither the spatial frequency specificity of the suppression effect (Fig. 1) nor its restriction to near-vertical orientations of the gratings is, however, characteristic of binocular rivalry observed during our experiments. Other experiments also indicate that the grating adaptation effect is not modified by binocular rivalry[11].

We thank Dr G. J. Burton for helpful discussion and interferometric equipment. We also thank Mr R. Dowdney for help.

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Suckling stimulation induces aggression in virgin female mice

THE aggression shown by parturient mammals of various species towards strange conspecifics[1,2], referred to as "maternal aggression", ostensibly serves to protect the young. Although much is known about the aetiology of other forms of aggressive behaviour, no information is available about the mechanism responsible for maternal aggression. We found recently that suckling stimulation may be important in the initiation of aggressive behaviour in parturient mice[3]. Animals whose nipples were removed before parturition and that were given foster young each day after delivery did not become aggressive. Moreover, we could not induce aggression in virgin female mice by exposing them continuously to foster young. A reason for this may be that virgin females, because of the lack of nipples, which usually develop during pregnancy, did not receive suckling stimulation from foster young. We hypothesised that if nipple growth were induced in virgin females, they would be able to receive suckling stimulation and thus would exhibit aggressive behaviour comparable with that of parturient animals. The results presented here confirm the hypothesis by showing that suckling stimulation induces aggression.

Eighty-five 60–65-d-old virgin female Rockland–Swiss mice (RS) were divided into four groups. One group of 24 mice, after bilateral overiectomy under ether anaesthesia, received a regimen of hormones that we had found to induce nipple growth. We gave 19 daily subcutaneous injections of 0.02 μg oestradiol benzoate dissolved in 0.02 ml sesame oil and 500 μg progesterone in 0.02 ml oil. (The synergistic action of oestrogen and progesterone in mice is confirmed by the fact that little or no nipple growth occurs in response to injections of either oestrogen or progesterone.) Another group of 20 virgin females was ovariectomised and administered oil for 19 d, while another 19 mice were sham ovariectomised and treated daily for 19 d with oil. A group of 22 mice was left intact and mated with RS males. Each animal was housed singly in an 11 × 7 × 5 inch polypropylene cage, the floor of which was covered with pine shavings. The animals had free access to food and water and were maintained on a 12 h light/12 h dark cycle with lights on between 0600 and 1800. Testing was between 0700 and 0900 and injections were given between 1000 and 1100.

The virgin mice were tested for aggression 24 h after the final injection of oil or hormone. Animals permitted to mate were tested on day 19 of gestation. An adult RS male was placed into the cage of each animal and left for 3 min or until a fight was observed. A fight was defined as 20 s of continuous biting and chasing. Immediately after the aggression test, each

Table 1 Aggressive behaviour of different groups of females

Group	*n*	No. fighting before presenting foster young	Proportion (and %) of animals fighting after pup exposure	Latency to begin fighting after pup exposure (d)	Average no. of fights*†
Experiment 1					
Parturient	22	0	12/22 (54)	1.8±0.8	—
OVX+EB+P	24	0	12/24 (50)	1.3±0.6	—
OVX+Oil	20	0	1/20 (05)	1.0	—
S–OVX+Oil	19	0	1/19 (05)	2.0	—
Experiment 2					
Parturient–THEL	20	0	4/20 (20)	1.3±0.4	9.8±0.4
Parturient–S–THEL	20	0	13/20 (65)	1.6±1.1	8.6±2.1
OVX+OB+P–THEL	20	0	2/20 (10)	2.5±1.5	8.0±1.0
OVX+OB+P–S–THEL	20	0	12/20 (60)	2.1±2.4	8.2±2.5

*Animals in experiment 1 were killed after they fought in two successive tests.

†Animals in experiment 2 were given 10 aggression tests. The data are derived from only the animals that fought.

Experiment 1: aggressive behaviour of RS female mice that were either mated (parturient), ovariectomised and primed with oestradiol benzoate and progesterone to induce nipple growth (OVX+OB+P), ovariectomised and treated with oil (OVX+Oil), or sham ovariectomised and given oil (S–OVX+Oil). Aggressive behaviour was scored before exposure to pups (on the last day of hormone or oil treatment or on day 19 of gestation) and on each succeeding day for 10 d after presentation of foster young or until fights were displayed on 2 successive days. Experiment 2: aggressive behaviour of RS females that were mated and either thelectomised (parturient–THEL) or sham thelectomised on day 19 of pregnancy (parturient–S–Thel) and of ovariectomised hormone-treated mice either thelectomised (OVX+OB+P—THEL) or sham thelectomised (OVX+OB+P–S–THEL) on the day after the last hormone injections.

animal was presented with five 3–8-d-old RS foster young. (The pregnant animals were allowed to deliver and their young were removed before presenting them with foster young.) On the next morning each animal was observed for maternal behaviour for 15 min, after which a test for aggression was conducted. Immediately after the aggression test the nipples of each animal were examined (red and distended nipples indicated that the female had received suckling stimulation) and the pups were removed and replaced by five other 3–8-d-olds. This procedure was continued for a further 9 d or until an animal exhibited aggression on two successive tests. The animals were killed at the termination of testing to determine whether milk was present in the mammary tissue.

Every animal exhibited the complete repertoire of maternal behaviour including retrieval of pups, licking of the pups' ano-genital region, and the assumption of the nursing posture. This confirms reports that non-pregnant and non-lactating mice display maternal behaviour[4].

None of the animals fought when tested before exposure to foster young (Table 1). Instead, they sniffed and groomed the male intruder. After exposure to young, 12 of 22 parturient mice and 12 of 24 hormone-treated virgin females were aggressive. The absence of aggression in approximately 50% of the animals in each of these groups is not unusual for we consistently find that 40–50% of parturient RS females are not aggressive[5]. In contrast, only one of 20 oil-treated, ovariectomised and one of 19 oil-treated, sham-ovariectomised virgins fought. Both oil-treated groups differed significantly from the hormone-treated animals and from the parturient animals with respect to the number that were aggressive (χ^2, $P < 0.01$).

Aggression typically occurred on the first test after exposure to pups, although a few animals began to fight on the second or third test. The topography of the fighting behaviour of the hormone-treated virgins was indistinguishable from that of the parturient animals. The initial latency to attack averaged 4 s with the attacks consisting of bites to the neck and flanks of the intruder male. Tail rattling and ano-genital sniffing were observed during fights but not before the initial attack. Intruders, never initiating a fight nor fighting back in response to attack, generally assumed submissive postures.

Daily inspection of the parturient and hormone-treated animals after exposure to pups revealed red and distended nipples. In both groups the pups attached themselves to the nipples such that they could maintain contact with the adult when the latter was lifted by the tail. In contrast, red and distended nipples and attachment were never observed in either of the groups injected with oil. Only the mammary glands of the parturient animals contained milk.

To verify further that the hormone regimen induced nipple growth, six more animals were placed into each treatment group. They were killed either after 19 d of hormone or oil treatment or on day 19 of gestation. The results are summarised in Table 2. On the average, nipple length was 100% and the base diameter was 30% greater for hormone-treated than for oil-treated virgin females. For pregnant animals, the same measures were 200% and 50% greater than those of oil-treated virgins.

Table 2 Average length (mm) and base diameter of a representative nipple of different animals

Group	Average length	Base diameter
Parturient	1.40±0.05*†	0.66±0.06*†
OVX+OB+P	0.99±0.07*	0.55±0.14*
OVX+Oil	0.49±0.08	0.43±0.08
S–OVX+Oil	0.47±0.13	0.41±0.06

Animals were either mated (parturient), ovariectomised and hormonally primed with oestradiol benzoate and progesterone (OVX+OB+P), ovariectomised and treated with oil (OVX+Oil), or sham ovariectomised and given oil (S–OVX+Oil).

*Significantly different (Fisher's least significant difference test, $P < 0.05$) from OVX+Oil and S–OVX+Oil.

†Significantly different from OVX+OB+P.

The findings show that virgin female mice can be induced to show aggression comparable with that of parturient animals by producing nipple growth and presenting them with young. Hormone exposure did not induce fighting directly, for treated animals were never aggressive before exposure to young. Suckling stimulation, therefore, may be essential for the initiation of aggression. To corroborate this we assessed the effect of the suckling stimulus.

Eighty 60–65-d-old virgin female RS mice were divided into four equal groups. Two groups were ovariectomised and given the hormone regimen described above. The nipples of one group were then removed (thelectomy) under ether anaesthesia on the day after the last hormone injection. The other group was sham thelectomised: 1-mm incisions were made along the ventral midline. The two remaining groups were left intact and mated with RS males. One of these groups was thelectomised on day 19 of pregnancy and the other was sham thelectomised. Testing and fostering were as before, except that animals were allowed to complete all 10 aggression tests.

All animals were comparable with respect to maternal behaviour. None was aggressive before exposure to young (Table 1). After exposure to pups, 13 of 20 sham-operated parturient mice and 12 of 20 sham-thelectomised hormone-treated virgins were aggressive compared with four of 20 thelectomised parturient animals and two of 20 thelectomised hormone-treated virgins. The thelectomised groups differed significantly from their sham-operated counterparts with respect to the number that fought ($P < 0.02$). The sham-thelectomised groups did not differ in terms of time taken to become aggressive or the average number of fights exhibited. Daily examination of both sham-operated groups revealed red and distended nipples, indicating that they had received suckling stimulation.

Thus hormone-treated virgins respond to thelectomy in a manner identical to that of parturient mice with thelectomy, significantly reducing the proportion of animals that are aggressive. The data agree with others which demonstrate the attenuating effects of thelectomy on maternal aggression in parturient mice[3].

Finally, 12 hormone-treated virgins and 12 parturient animals that fought the intruder on the third day of exposure to foster young were deprived of their foster young immediately after the fighting test and were retested 24 h later. If fighting did not occur on retesting, five foster young were placed into the cage and, beginning 24 h later, daily aggression tests and daily fostering of young were carried out until fighting recommenced. Animals that fought 24 h after removal of foster young continued daily aggression tests in the absence of the young. As soon as fighting was no longer exhibited, foster young were reintroduced and daily tests were conducted until fighting began.

All animals stopped fighting after pups were removed and resumed fighting when they were replaced. The hormone-treated virgins and the parturient animals did not differ in terms of the number of days after removal required to terminate fighting (range = 1–4 d, median = 1.0 d) or the number of days of pup replacement required to re-establish fighting (all within 1 d). Each animal had red distended nipples and nipple attachment after replacement of foster young. Thus the aggression of both hormone-treated and parturient mice can be eliminated by removing the suckling stimulus and re-established by reintroducing that stimulus.

The fighting behaviour induced in female mice hormonally primed to induce nipple growth could have been a response to chronic stress, produced by chronic suckling by deprived young, rather than a response specific to the suckling stimulus. We do not believe that this is the case, however, because virgin females and lactating mice whose pups had been removed did not attack a male after 24 h of intermittent electric shock to the paws. Moreover, virgins and lactating mice deprived of young did not attack a male after chronic application of painful stimulation to the nipples. Thus fighting in hormone-treated virgin female mice seems to be a response specific to the suckling stimulus.

Our data show that suckling not only supplies nourishment to the young but also affects the relationship between the mother and other adult members of her species. Suckling stimulates the release of hypothalamic and pituitary hormones[6], alters the rate of synthesis of hypothalamic monoamines[7], and changes the firing patterns of hypothalamic neurones[8]. One or more of these changes may induce aggressive behaviour in lactating mice.

This research was supported by grants from the US Public Health Service.

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Received November 7, 1975; accepted January 9, 1976.

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Transmission of *Trypanosoma rotatorium* from frogs to white mice

ALTHOUGH the trypanosome *Trypanosoma rotatorium* Mayer 1843, lives in the blood of frogs (*Rana esculenta* and *R. temporaria*) it does not have a pathogenic effect on them. Since no information is available about the mass incidence of these parasites in the blood of homoeothermic animals, we have tried to infect white mice with *T. rotatorium* in order to study the development of infection in these hosts.

Our experimental group consisted of 100 male and female white laboratory mice, strain ICR, from 3 weeks to 3 months old, which had been standardised both microbiologically and parasitologically (specific pathogen-free). Blood from young frogs (0.4–0.6 ml) diluted in 0.9% NaCl was administered intraperitoneally so that the final infective dose was 500 trypanosomes per animal. For controls we used 50 mice aged 3 weeks to 3 months which were given blood from uninfected frogs diluted in saline (2:1 v/v); 25 of them were injected with 2.5 mg of hydrocortisone twice a week for 3 weeks. The criterion for termination of incubation was the first appearance of trypanosomes in the peripheral blood of the mice. Dry blood smears were stained by the standard Giemsa method.

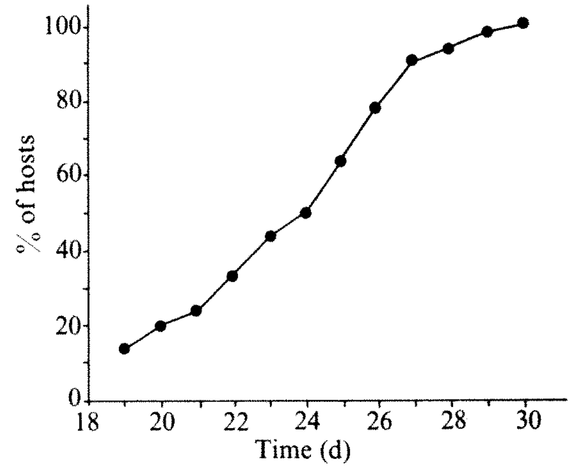

Fig. 1 The first incidence of *Trypanosoma rotatorium* in the blood of the individual mice is dependent on time.

Infection developed slowly with an incubation period of 18–30 d. There was considerable variation in the first appearance of trypanosomes in individual mice (Fig. 1). On day 24 of infection, trypanosomes were present in the blood of 50% of mice, 6 d later they were present in all hosts (100 mice).

The slender body of the trypanosomes, 10.6–18.7 μm long and 1.3–5.0 μm wide, ended in a short, obtuse rostrum. The oval nucleus was in the middle of the body and the blepharoplast was near its end. The undulating membrane passed into a short, free flagellum whose length did not exceed one-tenth of the body length (Fig. 2). Numerous dividing trypanosomes appeared in the blood of the mice. Division was binary and of the standard type. Multiple division in crithidia was not observed (Fig. 3).

No overt signs of disease were evident before the first appearance of trypanosomes in the blood of infected animals. An increase in the number of blood parasites was manifest in

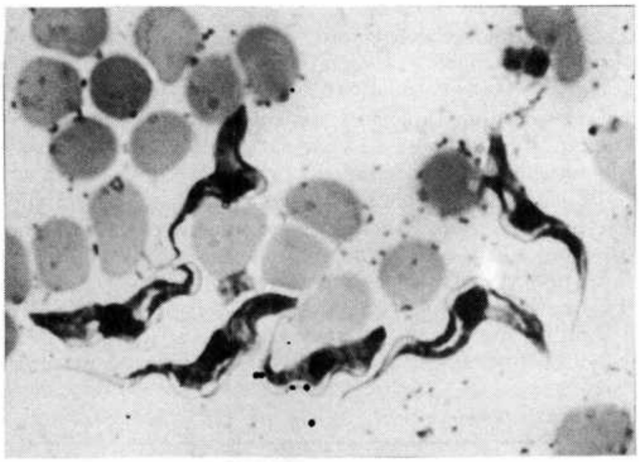

Fig. 2 *T. rotatorium* in the blood of a white laboratory mouse. Dry smear stained with Giemsa (× 1,600).

loss of appetite, loss of weight, bristling of the fur and general restlessness. The animals died within 2–5 d of multiplication of the parasites in the blood (maximum incidence 100,000 trypanosomes per ml of blood).

Since no information was available about transmission of trypanosomes from poikilotherms to homoeotherms our findings with mice had to be compared with published data on parasites from tadpoles and frogs[2-6]. During the development of infection in the frog, the morphology of trypanosomes changes considerably[2-6]. Trypanosomes from tadpoles and young frogs are slender, with a long flagellum[5,6]. Those multiplying in the blood of mice, however, reached about half the size of those multiplying in frog blood.

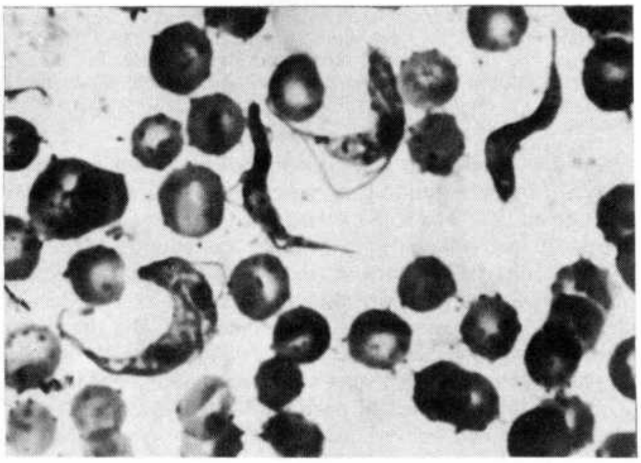

Fig. 3 In the left corner at the bottom *T. rotatorium* dividing in blood of white mouse. Stained with Giemsa (× 1,600).

A form of trypanosome that is often described from the blood of adult frogs is large and almost immobile, with a striped pellicle and a short undulating membrane which does not continue in a free flagellum[2,4-6]. This form has never been found in mice.

Compared with *T. duttoni*, one of the most frequent trypanosomes of rodents[1,7], *T. rotatorium* from mice was more slender, and its rostrum was shorter. There were differences in the location of the blepharoplast which is near the nucleus in *T. duttoni*, and the length of the free flagellum which reaches a half to two thirds of the body length of *T. duttoni*. In contrast to *T. duttoni*, *T. rotatorium* never produced multiple forms in mouse blood[1,7].

Thus the morphology of trypanosomes from mice is closest to that of trypanosomes from tadpoles and young frogs, except for the larger body size of those in the poikilothermic hosts and the very short free flagellum of those in mice.

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Received December 16, 1975; accepted February 25, 1976.

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Study of haemopoietic microenvironment *in vitro*

THE concept of the haemopoietic inductive microenvironment as first proposed by Curry and Trentin[1] has been amply confirmed by experiment. One salient feature of this concept relates to the role of the mouse spleen in promoting erythropoiesis rather than granulopoiesis. For example, in the clonal growth of transplanted bone marrow cells, the spleens of the irradiated host mice have erythroid and granuloid colonies in a ratio of 3, whereas the ratio is 0.5 in the bone marrow of the same hosts[2,3]. Other data show that there is a positive effect of splenic tissue, rather than a negative effect of bone marrow, on erythroid colony growth[3]. We have tested this concept using cocultures of mouse spleen and bone marrow cells and have found that

Table 1 Effect of NS and XS cells on marrow erythropoiesis

	Haemoglobin ^{59}Fe (±s.d.) (c.p.m.)	Δ c.p.m.	% of marrow control
Marrow cells − E	17±6	—	100
Marrow cells + E	232±16	215	1,260
NS cells − E	3±2	—	18
NS cells + E	17±3	14	100
Marrow + NS cells − E	50±10	—	294
Marrow + NS cells + E	286±45	236	1,680
XS cells − E	0	—	0
XS cells + E	0	0	0
Marrow + XS cells − E	150±11	—	880
Marrow + XS cells + E	491±18	341	2,890

E, erythropoietin. Cells were cultured in 0.2 ml samples, as previously described[6], in the wells of culture dishes. The medium, *p*H 6.9, consisted of 65% NCTC-109 containing 30 mM morpholinopropane sulphonic acid (MOPS), 30% foetal calf serum, 5% mouse serum containing 73 nmol of ferric nitrate per ml, and gentamicin (0.5 mg ml^{-1}). Both the foetal calf serum and the mouse serum were heat-inactivated at 56 °C for 30 min before use. Six replicate cultures were established per group. A sheep plasma preparation of erythropoietin (16 mU) with a potency of 200 U per mg of protein was added to the appropriate cultures dissolved in 1 µl of 0.1% bovine serum albumin, 0.15 M NaCl. The wells were sealed with sterile plastic adhesive sheets. After 20 h of incubation at 37 °C the wells were opened and 20 µl of ^{59}Fe-labelled mouse serum (0.2 µCi) were added to each; the wells were then resealed and incubated for 5 h, after which incorporation into haemoglobin was measured as before[6,7]. Spleens were removed aseptically from 10–14-week-old BDF$_1$ female mice. XS cells were prepared from mice which had received 900 R of whole-body X rays 3 d before use. Marrow cells were cultured at 2.9×10^6 per well; NS and XS cells were cultured at 2.7×10^6 per well. Spleen cells were dispersed by forcing splenic tissue through a sterile stainless steel screen (60 mesh) into 70% NCTC-109, 30% foetal calf serum. Remaining cell clumps were broken up by repeated passage of the suspension through a 26-gauge needle. The cells were washed once in phosphate-buffered saline before appropriate dilution in complete medium. Essentially the same results were obtained when the spleen cells were dispersed by digestion with collagenase.

Table 2 Effect of freezing and thawing of irradiated spleen cells

	Haemoglobin ^{59}Fe (±s.d.) (c.p.m.)	Δ c.p.m.	% of marrow control
Marrow cells − E	5±3	—	100
Marrow cells + E	112±15	107	2,240
XS cells − E	0	—	0
XS cells + E	0	0	0
Marrow + XS cells − E	52±2	—	1,040
Marrow + XS cells + E	197±14	145	3,040
Frozen-thawed XS cells − E	0	—	0
Frozen-thawed XS cells + E	0	0	0
Marrow + frozen-thawed XS cells − E	19±3	—	380
Marrow + frozen-thawed XS cells + E	85±14	66	1,700

Culture conditions and procedure were as outlined in Table 1. In the preparation of frozen and thawed XS cells, the cells in 25 μl of complete medium were added to appropriate wells and the dishes were placed on a mixture of crushed dry ice and ethanol for 15 min. They were then incubated at 37 °C for 20 min before the bone marrow cells were added in 175 μl of complete medium. Marrow cells were cultured at 3.2×10^6 per well; XS cells were cultured at 1.7×10^6 per well.

phenomena related to the haemopoietic inductive microenvironment can be studied *in vitro*.

Table 1 shows that spleen cells in culture augment haemoglobin synthesis by marrow cells *in vitro* in two ways. These may be characterised as an effect on baseline haemoglobin synthesis and an effect on erythropoietin-induced haemoglobin formation. Cells from the spleens of normal mice (NS cells), as well as from the spleens of mice given 900 R of whole-body X radiation 3 d earlier (XS cells), caused increased incorporation of ^{59}Fe into haemoglobin when they were added to marrow cell cultures. The effect on the baseline was almost three times greater with XS than with NS cells. Neither NS nor XS cells alone synthesised significant amounts of haemoglobin by the method used. In addition, XS cells but not NS cells caused a significant potentiation of the marrow cell response to erythropoietin, amounting to about 60% more stimulated haemoglobin synthesis than found with marrow cells alone. The NS cells showed a slight response to erythropoietin, but XS cells never did.

We attempted to determine whether intact XS cells were required for the observed effects. The data in Table 2 show that freezing and thawing of the cells reduced but did not abolish the effect on the baseline, and completely eliminated the effect on erythropoietin-induced haemoglobin synthesis. In fact, the frozen-thawed preparation caused a 38% suppression of the response to erythropoietin (Δ c.p.m.). This suppression is significant at the $P<0.02$ level.

Table 3 Effect of further irradiation of spleen cells on marrow haemoglobin synthesis

	Haemoglobin ^{59}Fe (±s.d.) (c.p.m.)	Δ c.p.m.	% of marrow control
Marrow cells − E	8±1	—	100
Marrow cells + E	77±5	69	963
XS cells − E	0	—	0
XS cells + E	0	0	0
Marrow + XS cells − E	40±3	—	500
Marrow + XS cells + E	155±7	115	1,940
XXS cells − E	0	—	0
XXS cells + E	0	0	0
Marrow + XXS cells − E	40±2	—	500
Marrow + XXS cells + E	170±11	130	2,120

Culture conditions and procedure were as outlined in Table 1. The XXS cells were prepared by irradiation of a sample of the XS cell suspension with 10,000 R of X rays just before culturing. There were 3.2×10^6 marrow and 1.8×10^6 spleen cells per well.

Additional X irradiation of XS cells *in vitro* with 10,000 R (XXS cells), immediately before they were added to the marrow cell cultures, enhanced the response to erythropoietin to a significantly greater extent ($0.02>P>0.01$) than did the single *in vivo* irradiation (Table 3). The effect on baseline haemoglobin synthesis, however, was the same with both XS and XXS cells, amounting to five times more incorporation.

The two effects (enhanced baseline synthesis and potentiation of response to erythropoietin) were distinguished further when species specificity was studied. Table 4 shows that baseline haemoglobin synthesis by rat bone marrow cells was increased 49% by mouse NS cells and almost 200% by mouse XS cells. In contrast, the response of rat marrow to erythropoietin was not affected by mouse NS cells and was depressed by mouse XS cells.

Table 4 Effect of mouse spleen cells on rat marrow erythropoiesis

	Haemoglobin ^{59}Fe (±s.d.) c.p.m.	Δ c.p.m.	% of marrow control
Rat marrow cells − E	47±11	—	100
Rat marrow cells + E	182±14	135	387
Mouse NS cells − E	0	—	0
Mouse NS cells + E	0	0	0
Rat marrow + mouse NS cells − E	70±7	—	149
Rat marrow + mouse NS cells + E	202±15	132	430
Mouse XS cells − E	0	—	0
Mouse XS cells + E	0	0	0
Rat marrow + mouse XS cells − E	140±9	—	298
Rat marrow + mouse XS cells + E	226±18	86	481

Culture conditions and procedure were as outlined in Table 1, except that bone marrow cells from male Sprague–Dawley rats (250–275 g) were used and rat serum was substituted for mouse serum. Marrow cells were cultured at 3×10^6 per well; NS and XS cells were cultured at 1.8×10^6 per well.

The data presented here permit several conclusions with respect to an understanding of the haemopoietic inductive microenvironment. (1) The role of the spleen in promoting erythropoiesis of mouse bone marrow cells can be studied *in vitro*. (2) Two different mechanisms seem to augment haemoglobin synthesis by splenic tissue. One concerns the existing level of haemoglobin formation by normal marrow cells; the second concerns the increase in response to added erythropoietin. (3) The action of normal spleen cells on baseline haemoglobin synthesis can be duplicated partially by cells which have been frozen rapidly and thawed, making it likely to be an effect of a cell constituent rather than of viable cells. (4) Mouse spleen cells can affect the baseline haemoglobin synthesis of rat marrow cells. (5) The action of either normal spleen cells or cells from the spleens of irradiated mice potentiates the action of erythropoietin on haemoglobin synthesis, but this effect is restricted to intact cells and may be species specific. Although it might be argued that the extent of erythropoietin-stimulated haemoglobin synthesis is maximal when rat marrow cells are incubated with mouse XS cells, so that an increase in the baseline will cause a necessary decrease in the erythropoietin effect, we have seen, in other experiments, much greater induced haemoglobin synthesis. This observation makes it more plausible that the decrease in c.p.m. from 135 to 86 (Table 4) represents a true suppression of erythropoietin action.

The nature of the cell constituent postulated above to be responsible for the action of spleen cells on baseline haemoglobin synthesis is unknown, and will be investigated further. Our evidence suggests that the second of the effects, that of potentiation of the response to erythropoietin, is mediated by intact cells. Bessis and Breton[4] have described a large cell, resembling a macrophage, around which erythroblasts are clustered. They have proposed that this cell acts as a "nurse cell" in normal erythropoiesis by facilitating iron transfer to the developing erythroblasts. This type of cell might be responsible for the effect we describe. Alternatively, the effect might be due to the type of radio-resistant cell that is found in spleen and is involved in the processing of antigen in the immune response[5].

We thank Nancy Pech for technical assistance. This work was supported in part by grants from the National Cancer Institute. G. E. v. Z. holds a National Institutes of Health postdoctoral research fellowship. The Franklin McLean Memorial Research Institute is operated by the University of Chicago for the US Energy Research and Development Administration under contract.

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Received December 18, 1975; accepted February 17, 1976.

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Several proliferative phases precede maturation of IgG-secreting cells in mitogen-stimulated cultures

SEVERAL workers have shown that stimulation of mouse spleen lymphocytes by *E. coli* lipopolysaccharide (LPS) results in extensive proliferation of B lymphocytes and the rapid maturation of immunoglobulin M (IgM)-secreting cells[1–3]. Not all B lymphcytes that are stimulated to DNA synthesis and division by LPS, however, develop into high rate Ig-secreting cells[4,5]; dividing blast cells can give rise to large numbers of non-dividing small B lymphocytes with a high density of surface Ig and no detectable intracellular Ig pool[4]. In these respects such secondary cells are thought to resemble memory cells. Those cells which do develop into high rate Ig-secreting cells during a few days of culture are generally restricted to secretion of IgM[7,8]. We report here that in improved culture conditions, and dependent on repeated cycles of mitogenic stimulation, precursors to IgG and IgA as well as IgM-secreting cells can undergo the full maturation sequence. Our results suggest that one or more phases of resting cells are intermediates in this sequence.

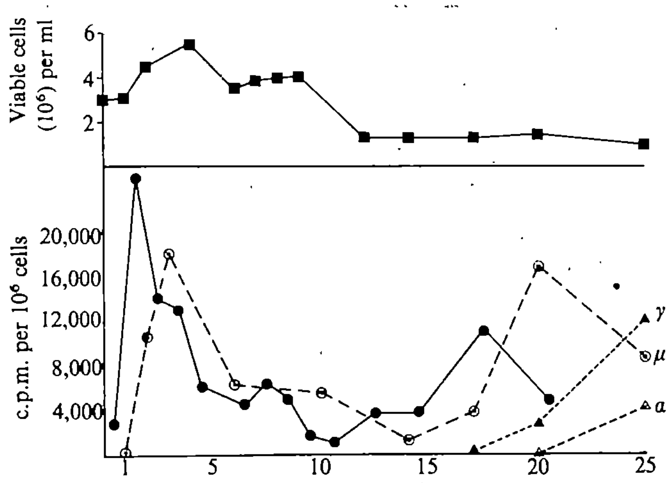

Fig. 1 DNA synthesis and Ig secretion in LPS-stimulated cultures. Spleen cells (CBA mice) were suspended, washed once and cultured at 3×10^6 cells per ml in RPMI 1640 medium (Biocult) supplemented with LPS (*E. coli* 055B, Difco, 10 μg ml^{-1}) 10% foetal calf serum (Flow), antibiotics and 6×10^{-5} M 2-mercaptoethanol (2ME). Culture dishes (60 mm) containing 5 ml were kept stationary at 37 °C in an atmosphere of 10% CO_2, 7% O_2 and 83% N_2. Every 3 d cells were sedimented (5 min at 240*g*) and resuspended in fresh, full medium and LPS. For ^{3}H-thymidine incorporation, duplicate 0.2 ml samples from the above cultures were incubated for 24 h at 37 °C with 1 μCi ^{3}H-thymidine (50 mCi mmol^{-1}) in microplates. The cells were filtered on Whatman glass fibre circles (GP/A), and washed with phosphate-buffered saline, trichloracetic acid, ethyl alcohol and ether[3]. Assessment of thymidine incorporation on microscale is justified; 4-h pulses in more recent experiments yield analogous results. For Ig secretion, cells sedimented from 0.6 ml of culture were incubated for 4 h in 0.4 ml of RPMI medium (containing only 2.5×10^{-5} M methionine), 10% foetal calf serum, 6×10^{-5} M 2ME and 20 μCi ^{35}S-methionine (> 100 Ci mmol^{-1}). The medium was then diluted with phosphate-buffered saline and methionine (1 mg ml^{-1}), cells were removed and the supernatant was clarified by high speed centrifugation. Ig was precipitated from half the supernatant using a polyvalent rabbit antiserum to mouse IgM and IgG and carrier Ig[8,9]. Ag/Ab precipitates, washed twice, were reduced and analysed by polyacrylamide gel electrophoresis as described in Fig. 2.

Mouse spleen lymphocytes were stimulated with LPS in the conditions described in the legend to Fig. 1. It is important for the long term maintenance of these cultures that medium be replaced every 3 d. The increase and then decline in cell number as well as the pattern of ^{3}H-thymidine incorporation and Ig secretion during 25 d are shown in Fig. 1. A high rate of ^{3}H-thymidine incorporation is observed on days 2 and 3 and followed on day 7 and again on day 17 by additional peaks of incorporation. Increases in cell numbers on days 4 and 8 reflect these high thymidine incorporation rates. The rate of Ig secretion increases to a maximum on day 3, after which it falls to a relatively low level. By day 20 the rate of Ig secretion is again very high (Fig. 1).

We have analysed the class of Ig secreted at various times in culture by fractionation of the Ig heavy chains on sodium dodecyl sulphate (SDS)–polyacrylamide gels.

Table 1 Induction of IgG secretion in T-cell depleted spleen

Spleen donor		Radioactivity (c.p.m. ml^{-1})†			Viable cells per ml ($\times10^{-6}$)	
		Day 19	28	31	19	28
Normal	μ Chains	8,470	38,060			
	γ Chains	2,730	20,620		7.0	1.5
T×B*	μ Chains	12,540	34,140	33,490		
	γ Chains	2,640	10,470	12,900	6.8	2.1

*(CBA×C57)F_1 mice were thymectomised, irradiated with 850 r. and reconstituted with foetal liver for 10 weeks. Spleen cells were treated with AKR anti-C_3H Thy. 1.2 and complement before culture[11].

†Radioactivity of secreted Ig heavy chains (^{35}S-methionine labelled) was analysed by electrophoresis after reduction of Ig precipitated with antiserum (see Fig. 2).

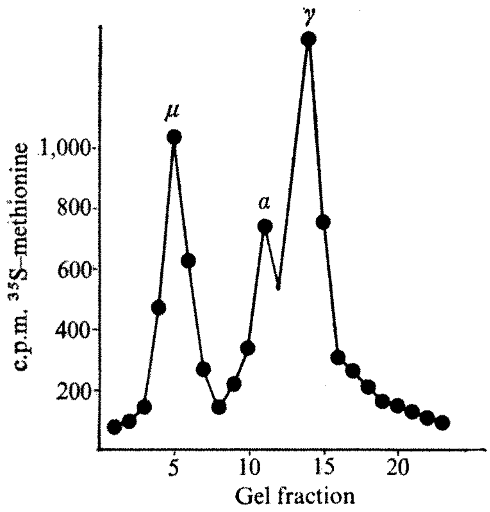

Fig. 2 Fractionation of secreted immunoglobulin heavy chains. ^{35}S-Ig secreted on day 25 (see legend to Fig. 1) was precipitated with antiserum to Ig, dissociated in 2% SDS, reduced overnight in 0.15 M 2ME at 37 °C in borate-buffered saline, and alkylated with iodoacetamide for 1 h at *p*H 8. Polyacrylamide gel electrophoresis was carried out as described by Maizel[10], gels were dried and then cut into 2-mm strips which were prepared for liquid scintillation counting by treatment with 0.5 ml H_2O_2 for 3 h at 70 °C. Internal markers for μ and γ chains were included on every gel and revealed by staining with Coomassie blue. In addition, labelled heavy chains of myeloma proteins confirmed the position of μ, γ and α chains.

A gel profile of reduced and alkylated Ig secreted into the culture medium during a pulse of ^{35}S-methionine on day 25 is shown in Fig. 2. Analysis on different days shows that only immunoglobulin of the IgM class is secreted early in the culture period, while late immunoglobulin secretion includes large amounts of IgG, some IgA and a new wave of IgM (Fig. 1). Cells fixed and stained for intracytoplasmic Ig using class-specific and fluorescent antisera (prepared by R. M. E. Parkhouse) have confirmed the presence of cells synthesising each of these Ig classes (unpublished results of M.Z. and R. M. E. Parkhouse).

Neither the activation of precursors nor the lag in maturation of the IgG-secreting cells depends on the presence of T cells, as shown by the similarity of results obtained using spleens from mice depleted of T cells, both *in vivo* and *in vitro*, or from normal mice (Table 1). The difference in amount of IgG secreted in the two types of culture is within the range of variation normally observed with different pools of spleen cells.

It has been shown[12] that the stimulation by antigen of cells producing specific IgG antibody in short term culture depends on the presence of a transient cell type which appears to be an intermediate between a long term memory cell and an antibody-producing cell. We have investigated whether this intermediate cell type responds to LPS and whether a transient pool of such cells in a normal spleen might account for the IgG response we have observed. Table 2 shows that these cells do respond to LPS and give comparable numbers of antibody-producing cells as in antigen-stimulated cultures. The peak of this response, however, is on day 5 and is easily differentiated from the larger LPS-stimulated IgG response detected by isotope incorporation and gel analysis on day 17. It is likely that this intermediate cell type is responsible for the reported[14,15] stimulation of a small number of IgG antibody-forming cells by mitogen in short term cultures.

Table 2 Specific anti-DNP-forming cells and IgG secretion

Additions	Day 5	7	10	13	17
	7S PFC/* culture				
None	530	20	20	20	—
DNP–KLH (0.1 μg ml^{-1})	3,360	1,080	170	40	0
LPS	2,300	300	620	545	30
	Secreted IgG (c.p.m. per culture)				
LPS	< 800	920	790	1,380	9,530

PFC, Plaque-forming cells. Radioactivity of secreted ^{35}S-methionine-labelled IgG was estimated as in Figs 1 and 2. Spleen cells were derived from mice primed and then boosted 8 d previously with DNP–KLH (dinitrophenol–keyhole limpet haemocyanin)[12]. Culture conditions were as described in Fig. 1.

* Specific indirect anti-DNP-forming cells were detected using horse red blood cells sensitised with trinitrobenzene sulphonic acid and then developing plaques with rabbit anti-mouse IgG[12,13].

The occurrence of a long lag period in LPS-stimulated cultures during which the level of ^{3}H-thymidine incorporation is low suggested that a non-proliferating cell might be an intermediate in the maturation of precursors to IgG and other late Ig-secreting cells. This was further investigated by the addition of bromodeoxyuridine (BUdR) to cultures at various times at a concentration (10 μg ml^{-1}) which on subsequent irradiation by means of a black lamp (300–400 nm), leads to the death of cells actively engaged in DNA synthesis. Only a few cells survived (less than 5%) when BUdR was present from 36 to 62 h after stimulation during the first wave of DNA synthesis, indicating that the dividing cells were killed. Precursor cells, however, did survive when a BUdR pulse was given on days 4 and 5 or 10 and 11, as shown by the maturation of large numbers of Ig-secreting cells later in the culture period (see below). It should be emphasised in this connection that stimulation of high rate IgG secretion depends on the re-addition of LPS to cultures when they are resuspended in fresh medium.

Table 3 Effect of BUdR on IgG secretion

Days	c.p.m. per ml culture* ^{3}H-thymidine	Cell no. per ml $\times 10^{-6}$	Ig† c.p.m. per ml culture μ chain	γ chain
Control 4	35,500	6.7	56,071	< 500
Control 7	9,250	3.0	54,417	4,455
Control 14		0.8	14,797	3,869
BUdR‡ 4	2,700	3.5	25,344	7,216
BUdR 7	48,250	1.9	4,356	< 440
BUdR 14		3.3	43,026	27,112

* 24-h pulse with 1 μCi ^{3}H-thymidine (50 mCi mmol^{-1}).

† Radioactivity of Ig secreted during a 4-h pulse with ^{35}S-methionine (see legends to Figs 1 and 2).

‡ BUdR (10 μg ml^{-1}) was added on day 3; after 24 h cells were collected and irradiated in the same volume as before in PBS for 20 min using a black lamp (Philips 20 W/08, 300–400 nm) before being resuspended in fresh medium. The same dishes were used throughout to avoid loss of adhering cells. Control cultures were handled in identical fashion in absence of BUdR.

An unexpected finding was that if BUdR is added and cultures are irradiated immediately after the first peak of ^{3}H-thymidine incorporation, precursor cells which survive initiate high rate IgG secretion and do so earlier than do controls. LPS-stimulated cells received a 24-h pulse of BUdR on day 3 of culture. ^{3}H-thymidine incorporation on day 4 immediately after the pulse is very low. By day 7, however, it has increased to a level many times greater than that observed in controls (Table 3). This effect is not due to a change in the specific activity of intracellular pools as a considerable increase in cell numbers occurs in parallel. Analysis of Ig secretion showed some early IgG (day 4) and large amounts of IgG on day 14. In these control cultures (washed and irradiated in the absence of BUdR) detectable IgG appeared earlier than usual (Table 3). Our interpretation of these experiments is that an inhibitory cell type which proliferates in LPS-stimulated cultures is eliminated by irradiation after the BUdR pulse. Secondary non-proliferating precursors quiescent during the pulse can then be activated sooner than would otherwise occur. Other experiments showed that cells present on day 9 of culture could block the activation of precursors to early IgM-secreting cells when mixed with freshly prepared spleen cells before, but not 27 h after, addition of LPS (M.Z., unpublished). Nevertheless, in view of the complexity of BUdR action[16,17], we cannot exclude the possibility that BUdR has a direct effect on precursor cells which speeds up further proliferation or leads to their rapid maturation. As all cells which are engaged in DNA synthesis are killed at the end of the pulse, this would have to be an effect on cells not proliferating during the pulse.

There have been two recent reports[5,18] that LPS treatment results, within 6–8 d, in the appearance of cells which synthesise IgG as detected by immunofluorescent staining of cytoplasmic Ig with specific antisera. In one case[5], these are described as immature, weakly staining lymphoblasts, while in the other[18], stronger staining denotes a more mature cell type in cultures initiated at low density. At such early times we detect low levels of IgG secretion in low density cultures set up according to Kearney and Lawton[18] and occasionally also in the conditions described here. High levels of IgG production, however, arise only late in our cultures and it is possible that several mitotic cycles are required before full maturation of these cells. The significance of initiating cultures at a low cell density is not clear, but may be a means of reducing inhibitory effects.

In conclusion, we have shown that precursors to IgG and IgA, as well as to IgM-secreting cells, can be activated by a mitogenic stimulus. After a proliferative phase they give rise to early IgM-secreting cells as well as to secondary non-proliferating cells. These secondary cells can be reactivated to undergo further proliferation and finally to mature to high rate IgG-, IgA- or IgM-secreting cells. An inhibitory cell type which also proliferates in these high density cultures may delay the reactivation of secondary cells. Neither the maturation of precursors to IgG production nor the inhibitory effects observed in LPS-stimulated cultures depends on the presence of T cells.

We thank P. Tilly for assistance and J. North for stimulating discussions.

Note added in proof: Additional evidence of production of IgG- or IgD-like molecules in low density cultures has been published[19].

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Received November 24, 1975; accepted February 26, 1976.

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Control of C3 levels in mice during ontogeny by a gene in the central region of the *H–2* complex

THE observation that differences in serum haemolytic activity in mice are controlled by the *H–2* complex[1], was followed by reports that in several mammalian species the levels of individual complement components (C), from both the classical and the alternative pathways, are influenced by genes located close to the major histocompatibility complex (MHC), (reviewed in ref. 2). The relationship between mouse Ss protein, serum levels of which are controlled by a gene in the central part of *H–2*, and human C4 (refs 3–5), highlights the association between MHC and the C system. The mechanism which underlies this association is largely unknown. In only one case (factor B) has a structural gene been identified[6]. We report here that the serum levels of C3 in mice during ontogeny are influenced by a gene in the central region of the *H–2* complex.

Breeding couples of inbred, congenic and congenic recombinant strains of mice were obtained either from commercial sources, or from Drs D. C. Shreffler and J. Stimpfling. Mice were bled in heparin-treated capillary tubes and plasma was stored at −70 °C. Antiserum to mouse C3 was obtained as before[7]. Immunoelectrophoretic analysis showed that this antiserum did not distinguish between native C3 and its degradation products C3b and C3d obtained by treating mouse serum with cobra venom factor. The specificity of this antiserum was ascertained by comparison with a monospecific antiserum to guinea pig C3 which cross reacted with mouse C3. In double diffusion in agarose against mouse serum, the lines given by the rabbit antiserum to guinea pig C3 and to mouse C3 coalesced in a reaction of identity. Simple radial immunodiffusion was performed as before[8]. Four different dilutions of a pool of normal mouse plasma were included in each plate as standards. Plates were incubated for 7 d at room temperature. When linear regressions were calculated for different plates for the relationship between areas of the rings compared with dilution of the standard antigen, coefficients of 0.999 or higher were obtained.

C3 levels in plasma of AKR/J mice were significantly higher than in DBA/2J until 4 weeks of age (ref. 8 and Table 1). At 9 weeks the differences were smaller and not significant. To assess the importance of the *H–2* complex in the control of C3 levels during development, segregation analysis and studies were performed with congenic and congenic recombinant mice which were 2 and 3 weeks old.

Table 1 summarises the results of segregation analysis, some of which have been reported already[8]. The 21-d-old progeny of the backcrosses (AKD2F$_1$ ♀ × DBA/2J or AKR/J ♂) or of the F$_2$(AKD2F$_1$ × AKD2F$_1$) mice were typed for *H–2* and classified as *k*/*k*, *d*/*k* or *d*/*d*. In all, 203 mice were examined. C3 levels were compared in all pertinent combinations and significant differences ($P<0.01$) in the expected direction were found in mice with different *H–2*. Furthermore, the differences in C3 levels between AKR/J and DBA/2J mice seem to be controlled by very few genes, for, with a single exception, there were no significant differences in C3 levels of mice with the same *H–2* type (Table 1). In ten comparisons, the only exception was that C3 levels of DBA/2J mice were significantly lower than those of the *d*/*d* progeny of F$_2$ mice. Measurements (not shown) of C3 levels at 15 d of age in the same backcrosses and F$_2$ showed an identical pattern of segregation. Body weights of mice with different *H–2* at 2–3 weeks of age were not different.

Table 2 shows that differences in C3 levels were detected among 21-d-old congenic mice of the B10 series which bear five unrelated *H–2* haplotypes. Plasma from 142 animals was analysed. $H{-}2^b$ and $H{-}2^d$ haplotypes are associated with small but significant differences in C3 levels. As expected from the results of the segregation analysis, $H{-}2^k$ mice have significantly higher C3 levels than $H{-}2^d$ mice. No differences were found between C3 levels of mice with $H{-}2^k$, $H{-}2^s$ and $H{-}2^f$ haplotypes.

The effect of allelic variation within the subregions of the *H–2* complex was studied in series of congenic and congenic recombinant mice with different S subregions of *H–2*. Each group consisted of a minimum of 20 mice, from three or more separate litters. Table 3 shows that C3 levels in A.AL strain were higher than in A strain. They differed only in the IC and S subregions. A.AL carries the *k* allele, and A carries the *d* allele in those subregions. In the C3H series, C3H.OH(S^d) and

Table 1 Segregation of C3 levels in serum of 21-d-old mice according to H–2

H–2 type	AKR/J	DBA/2J	F_1	$F_1 \times$ AKR/J	$F_1 \times$ DBA/2J	$F_1 \times F_1$	F^x_y
k/*k*	0.86±0.13 (29)	—	—	0.81±0.09 (8)	—	0.91±0.14 (12)	$F^2_{46} = 1.57$ NS
d/*k*	—	—	0.70±0.07 (8)	0.74±0.06 (17)	0.69±0.09 (14)	0.76±0.12 (45)	$F^3_{80} = 2.58$ NS
d/*d*	—	0.53±0.06 (36)	—	—	0.58±0.09 (12)	0.68±0.10 (22)	$F^2_{67} = 23.4$ $P < 0.01$
F^x_y	$F^2_{70} = 100$ $P < 0.005$			$F^1_{23} = 53$ $0.025 > P > 0.01$	$F^1_{24} = 9.9$ $P < 0.005$	$F^2_{76} = 15.0$ $P < 0.005$	

Numbers not in parentheses represent relative concentration of C3 in plasma (mean±s.d.). Numbers of animals are given in parentheses. The differences or similarities in complement levels between groups of strains, or multiple groups of animals, were estimated by analysis of variance. In the F^x_y ratios, *x* represents degrees of freedom for genetic variation and *y* represents degrees of freedom for phenotypic variation.

C3H.OL(S^k) strains, which differ only in the S subregion, have small but significantly different C3 levels in the direction predicted from the segregation studies. B10.Br and B10.M(17R) are identical in the K, IA and IB subregions, and have different C3 levels; B10.Br is *k* in the IC, S and D subregions, and has higher C3 levels than B10.M(17R) which is *d* in IC and S subregions.

These comparisons show that the gene controlling C3 levels is located within or closely linked to the S subregion of *H–2*. Some additional observations are compatible with this hypothesis. (1) In the A/WySn-A.CA combination, the *f* allele in the S subregion is associated with higher C3 levels than the *d* allele, and in the C3H series, the *d* and *k* alleles of S have higher C3 levels than the *b* allele. These findings agree with the effects of these alleles in the B10 series (Table 2). (2) C3 levels of five congenic and congenic recombinant strains of the B10 series, which have identical alleles in the IC and S subregions (IC^d, S^d), were also compared. B10.A, B10.A(2R), B10.A(17R), B10.D2 and B10.A(5R) differ in the K, IA and IB subregions (*k*, *b*, *d* and *f* alleles) and also in the D subregion (*d*, *b* and *f* alleles). Their C3 levels, however (0.87±0.12, 0.89±0.11, 0.85±0.09, 0.86±0.08 and 0.83±0.12, respectively), were not significantly different ($F^4_{138} = 2.0$).

Table 2 C3 levels in congenic mice with different *H–2* haplotypes

Congenic line	*H–2*	C3 levels
C57BL/10sn	*b*	0.81
B10.D2	*d*	0.86
B10.M	*f*	0.95
B10.Br	*k*	0.96
B10.S	*s*	0.98

H–2^b against *H–2*^{d} ($F^1_{63} = 6.8$; $00.5 > P > 0.01$). *H–2*^{b}, *H–2*^{k} and *H–2*^{s} against *H–2*^{b} and *H–2*^{d} ($F^2_{140} = 40.6$; $P < 0.001$). The differences between *H–2*^{k}, *H–2*^{f} and *H–2*^{s} are not significant.

C3 levels in newborn mice are also influenced by gene(s) outside the *H–2* complex. Indeed, at 3 weeks of age significant differences ($P < 0.01$) were observed between C3 levels of mice with the same *H–2* but different backgrounds such as DBA/2J (0.54±0.06) and B10.D2 (0.86±0.08); B10.M (0.95±0.08) and A.CA (0.81±0.05); C3H.B10 (0.94±0.2) and C57BL/10 (0.81±0.09).

Because genetic polymorphism of human C3 can be detected by electrophoresis[9] we subjected the plasma of mice with *H–2*^{k}, *H–2*^{d}, *H–2*^{b}, *H–2*^{s} and *H–2*^{f} haplotypes in different backgrounds (A, C57BL, C3H, DBA/2J and AKR/J) to high voltage electrophoresis in Agarose gel. No differences in the mobility of the C3 band were observed. We conclude that the levels of C3 in newborn mice are controlled by at least two genes, one of which is situated in the central region of the *H–2* complex, as shown by segregation analysis and by the studies on congenic and congenic recombinant mice. In adult mice, significant differences in C3 levels were no longer detectable. Perhaps these genes cease to function, or more likely, their influence is masked by the activity of other genes and/or environmental influences.

The nature of the *H–2* gene which controls C3 levels in ontogeny is unresolved. On the basis of preliminary mapping, it can be separated from the *Hom–1* gene[10] situated at the K end of *H–2* (ref. 11) which influences testosterone levels and the relative weights of several organs which are hormone-regulated. Meruelo and Edidin[12] have demonstrated that basal levels o

Table 3 C3 levels in congenic mice with different S subregion of *H–2*

Congenic line	K	IA	IB	IC	S	D	C3 levels	F^x_y	*P*
	H-2 subregions								
A/SfSn	*k*	*k*	*k*	*d*	*d*	*d*	0.82		
A.AL	*k*	*k*	*k*	*k*	*k*	*d*	0.97	$F^1_{62} = 32.1$	<0.001
C3H.B10	*b*	*b*	*b*	*b*	*b*	*b*	0.94		
C3H.OH*	*d*	*d*	*d*	*d*	*d*	*k*	1.05	$F^2_{61} = 6.5$	<0.005
C3H.OL*	*d*	*d*	*d*	*d*	*k*	*k*	1.18		
B10.Br	*k*	*k*	*k*	*k*	*k*	*k*	0.96		
B10.M (17R)	*k*	*k*	*k*	*d*	*d*	*f*	0.85	$F^1_{42} = 8.9$	<0.001
A/WySn	*k*	*k*	*k*	*d*	*d*	*d*	0.74		
A.CA	*f*	*f*	*f*	*f*	*f*	*f*	0.81	$F^1_{43} = 15.3$	<0.001

* The differences between C3 levels of C3H.OH and C3H.OL are significant ($F^1_{48} = 4.21$; $0.05 > P > 0.01$).

cyclic AMP in the liver of adult mice segregate with *H–2* in F_2 mice, and also differ significantly among relevant combinations of congenic mice. They also present evidence to suggest that two genes, one at each end of the *H–2* complex, are involved. But the precise position of these genes within the subregions of *H–2* has not been determined. If one of the cyclic AMP genes is mapped within or close to the S subregion, the possibility of its direct or indirect involvement in the control of C levels should be considered.

This work was supported by grants from the National Institutes of Health.

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Received January 20; accepted February 18, 1976.

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Microtubule assembly mutant

WE report here a new maternal effect mutation (*nc*, for no cleavage) in the Mexican axolotl, *Ambystoma mexicanum*. This mutation involves a defect in the assembly of microtubules in the fertilised egg. There is a large literature describing the molecular and biochemical events which accompany the *in vitro* self-assembly of microtubules; the *nc* mutant is the first known system with a genetic lesion in the regulation of these events *in vivo*. This mutant will provide a means to examine directly the control of microtubule assembly, and may also be useful in the identification of the events which trigger initiation of cleavage after activation of the egg at fertilisation.

Eggs spawned by female axolotls homozygous for *nc* seem to be morphologically normal. They show normal interaction with sperm, which enter and cause activation of the eggs as determined by the standard criteria of cortical changes, formation of polar bodies and swelling and migration of the pronuclei. But no mitotic apparatus or cytasters are assembled and no cleavage occurs. The mutant eggs do contain a pool of soluble tubulin indistinguishable from that in normal eggs. (A detailed description of the characterisation of axolotl tubulin will be published elsewhere.)

We have found that the lesion in the *nc* mutant eggs can be partially corrected by injection of microtubule fragments. If fertilised *nc* eggs (which are arrested after the cortical activation stage) are injected with fragments of the outer-nine doublet tubules prepared from sea urchin sperm tail axonemes, they begin to cleave and proceed to partial blastula as shown in Fig. 1. Cleavage begins at about the time it would have started had the eggs initiated cleavage normally. Normal fertile axolotl eggs cleave about 7 h after spawning at 18 °C; fertilised *nc* eggs injected with doublet tubule fragments 1 or 2 h after spawning begin to cleave 5–6 h later. The initial cleavages are fairly normal in appearance in the animal hemisphere but there is little cleavage in the vegetal region of the eggs. Later cleavages become more irregular, resulting in the formation of partial blastulae, and the eggs finally arrest. The doublet microtubule fragments which elicit cleavage are composed of tubulin; dynein and other high molecular weight proteins are absent (Fig. 2).

No cleavage results after injection of soluble tubulin, buffer, non-tubulin particles or normal germinal vesicle nucleoplasm. In addition, no cleavage results after injection of doublet tubule fragments into activated *nc* eggs followed by bathing the eggs in colchicine at a concentration which arrests cell division of normal fertile axolotl eggs. Activation of the egg is necessary for the correction effect: injection of microtubule fragments into unactivated infertile eggs from either *nc* mutants or normal females does not cause cleavage.

In some preliminary experiments, eggs were injected with basal bodies isolated from *Chlamydomonas reinhardi*. These preparations are much more heterogeneous than the doublet tubule fragments and the results were more variable and difficult to assess. A very small proportion of eggs injected with basal bodies underwent cleavage comparable with that caused by doublet tubules; most did not cleave at all, and some underwent abnormal cleavage or fragmentation, which is clearly distinguishable from cleavage.

The *nc* mutant eggs can be phenocopied by electrically stimulating infertile eggs from normal females. This causes

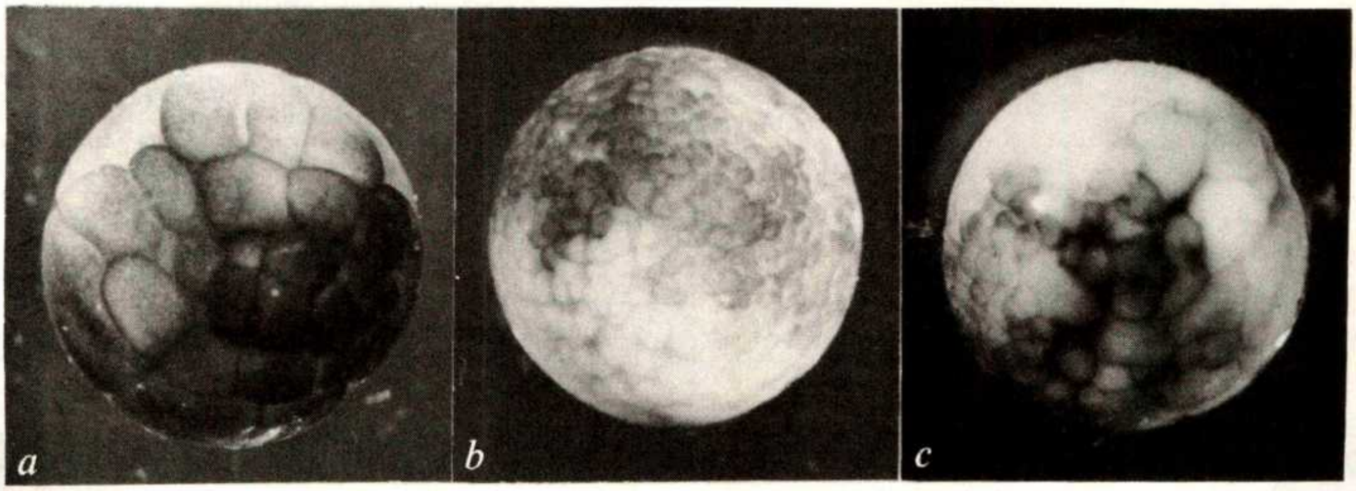

Fig. 1 Cleavage of normal and corrected *nc* mutant eggs. *a*, Normal fertilised egg in mid-cleavage. *b*, Mutant egg corrected by injection of doublet tubules. Cleavage has progressed in the animal hemisphere to form a partial blastula similar in appearance to a normal blastula. This is an example of the best correction observed. *c*, Mutant egg corrected by injection of doublet tubules. This is an example of the poorest correction observed containing patches of irregular sized cells.

Table 1 Induction of cleavage in *nc* mutant and normal axolotl eggs

Treatment	Mutant: activated *nc* eggs	Phenocopy: electrically activated normal eggs	Unactivated eggs: infertile *nc* or normal eggs
Injection of doublet tubule fragments prepared from sea urchin sperm tails	+*†	+	–
Injection of buffer	–*†	–	–
Injection of soluble tubulin prepared from *Drosophila* embryos	–*†	–	–
Injection of germinal vesicle nucleoplasm from normal eggs	–*	–	–
Injection of heat-killed bacteria (*Staphylococcus aureus*)	–*	–	–
Injection of doublet tubule fragments followed by bathing in 10^{-3} M colchicine‡ or 10^{-4} M vinblastine	–†	–	–

+, Initiation of cleavage; –, No cleavage.

* Activated by interaction with sperm (that is, fertilised eggs).

† Activated by electrical stimulation (of infertile eggs).

‡ This concentration of colchicine causes arrest of normally dividing fertile embryos. The relatively high concentration is necessary because of the impermeability of the eggs.

activation of the eggs so that morphologically they appear the same as fertilised eggs but they do not subsequently undergo cleavage. (Occasionally some of the electrically activated eggs fragment; these eggs are not used in correction experiments.) Electrically activated eggs also initiate cleavage to form partial blastulae when injected with doublet tubule fragments. Likewise, electrically activated infertile *nc* mutant eggs initiate cleavage when injected with doublet tubule fragments. A summary of the results of microinjection experiments is given in Table 1.

Preliminary light and electron microscopic observations of sections of the partial blastulae resulting from injected *nc* eggs show that the blastomeres are surrounded by complete cleavage furrows and that each blastomere contains an area in which the cytoplasm is cleared and in which parallel arrays of microtubules are formed. These arrays are occasionally but not usually organised into a shape resembling a true spindle. Chromosome fragments are found associated with these arrays in some (but not all) of the blastomeres. The initiation of cleavage thus appears to be due to a triggering of microtubule assembly from the existing pool in the egg, since the amount of tubulin contained in the injected fragments is less than 10% of the amount already present in the egg. In addition, in some cross sections we have observed doublet tubules, indicating that the injected fragments retain their morphological integrity.

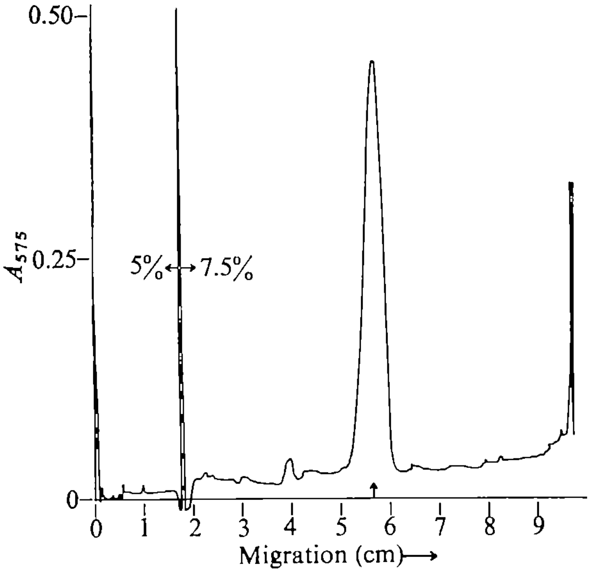

Fig. 2 Purity of injected doublet microtubule fragments. Doublet tubules were solubilised in 8 M urea–0.2% sodium dodecyl sulphate (SDS) buffer and electrophoresed on an SDS–urea polyacrylamide gel which was then stained with Coomassie blue and scanned. The running gel consisted of a 2-cm gel containing 5% acrylamide poured on top of an 8-cm gel containing 7.5% acrylamide. A stacking gel was poured over this. This allows good resolution of both high and low molecular weight sperm tail proteins. Tubulin (indicated by arrow) accounts for 97% of the protein present in the doublet tubule fragments used in our experiments. Dynein, which would be represented by a peak in the 5% gel, is absent from this preparation but is clearly visible in gels of whole axonemes.

It is our hypothesis that the lesion in the *nc* mutant lies in an early microtubule assembly event stimulated by activation, an event possibly analogous to the formation of mitotic organising centres in activated (but not in unactivated) eggs of the surf clam *Spisula*, as described by Weisenberg and Rosenfeld[1]. Although the correction obtained is not complete, in that the eggs do not finish embryonic development, nevertheless the normal timing of cleavage initiation and the presence of microtubule arrays and chromosome fragments in the blastomeres indicates that a process resembling normal cell division is elicited. One very intriguing aspect of the *nc* eggs is that during activation they seem to complete meiosis. We do not know whether this indicates a molecular difference in the meiotic and mitotic spindles or a difference in regulation.

Fraser achieved parthenogenetic development of *Rana pipiens* eggs by injection of crude fractions possibly containing microtubules[2]. Injection of axonemes from *Tetrahymena* or *Chlamydomonas* did not elicit cleavage; Heidemann and Kirschner[3] postulated that Fraser's active fractions contained centrioles. Ohta and Iwamatsu[4,5] achieved partial development of infertile eggs of the medaka by injecting them with flagella or microtubules prepared from sea urchin sperm. In both these organisms, unlike the axolotl, parthenogenetic development of infertile eggs is fairly readily induced and activation of the egg occurs during microinjection. Heidemann and Kirschner[3] demonstrated that injection of isolated basal bodies into infertile eggs of *Xenopus laevis* elicited both aster formation and initiation of a type of cleavage with irregular furrows and fragmentary blastomeres. This is similar to what we have scored in our experiments as fragmentation. These authors do not discuss the state of activation of the *Xenopus* eggs after injection of basal bodies. They found, however, that isolated flagella or axonemes were ineffective in inducing aster formation or cleavage when injected into infertile *Xenopus* eggs.

In the *nc* axolotl mutant there is a clear-cut separation between the events of activation and subsequent initiation of cell division. In this mutation, for the first time a genetic lesion has been found which involves the role of microtubule assembly in the initiation of cell division and subsequent embryonic development. A detailed description of this mutant will be published elsewhere.

We thank J. W. Brandis and J. E. Loyd for doublet tubules, L. H. Green for soluble tubulin, F. R. Turner for electron microscopy and L. Lawrence for photography. This work was supported by the US National Science Foundation.

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Received December 8, 1975; accepted February 12, 1976.

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Mechanical properties of vascular smooth muscle cells *in situ*

ALTHOUGH the histology and ultrastructure of smooth muscle cells have been studied extensively there is little direct evidence concerning their mechanical properties. Their small size[1,2] and lack of tendon connections have prevented them from being mounted on a myograph, as has been done with striated muscle fibres[3]. Moreover, their multiplicity and optical properties make it difficult to visualise them in whole smooth muscle preparations. Although isolated smooth muscle cells[4–6] can be seen to contract when stimulated, it has not been possible to measure the forces they develop. There are, therefore, still serious doubts about the extent to which the mechanical properties of whole smooth muscle preparations are an accurate reflection of the properties of smooth muscle cells. We have therefore developed a technique to obtain mechanical data from a smooth muscle preparation which only contains about 1,000 smooth muscle cells, and which is thin enough for the cells to be visualised by Nomarski interference microscopy[7]. This technique provides for the first time the means to study the mechanical properties of smooth muscle cells *in situ*.

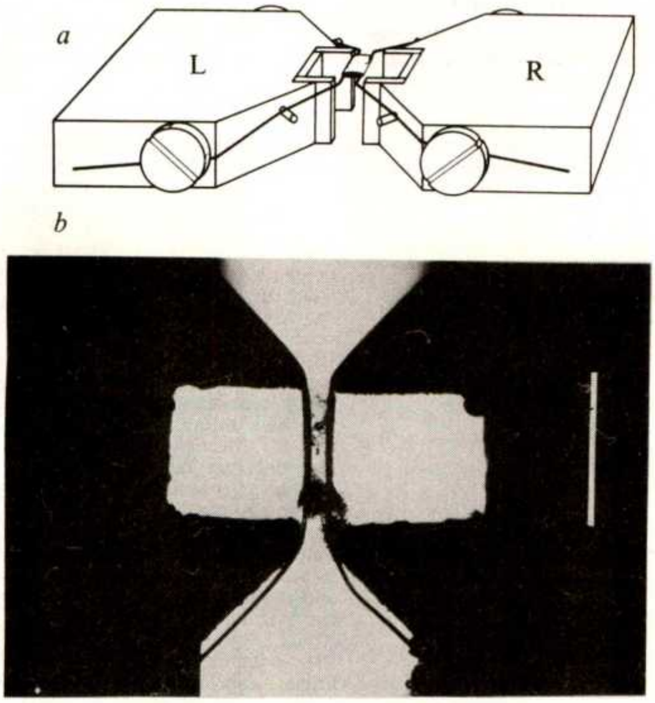

Fig. 1 *a*, Sketch showing method of mounting vessel segment. The vessels were excised from the animal together with about 1 cm of the proximal arterial tree. The latter was threaded on to a 32-μm tungsten wire and used to guide the wire into the lumen of the test vessel. The unwanted tissue was then dissected away and the wire was clamped at each end under tension to the left-hand support (L). A second wire was then passed through the lumen and similarly attached to the right-hand support (R). The supports were mounted respectively on a tension transducer (DSC 6, Kistler-Morse, resonant frequency 400 Hz with support mounted) and a piezoelectric vibrator (PZ40, Burleigh, range 19 μm, frequency response 0–2 kHz). The vibrator was in turn mounted on a micrometer translator for gross movement. Movement of R was detected using an eddy-current displacement transducer (KD 2300.5 SU Kaman). The supports were immersed in a chamber through which solutions were circulated. *b*, Low power micrograph of a vessel when mounted as described in (*a*) and viewed from above. The bar shows 1 mm.

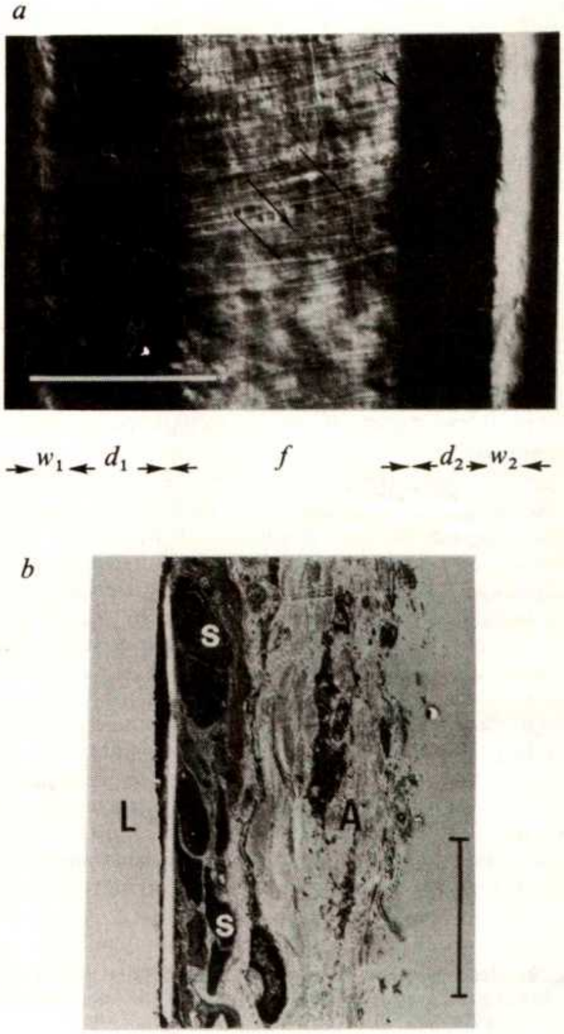

Fig. 2 *a*, High power micrograph of the vessel shown in Fig. 1*b* with focal plane in the media of the upper wall. The general outline of the smooth muscle cells is seen (the long arrows show a number of cell boundaries). The two dark areas (short arrows) are the 32-μm mounting wires. Photographed using Nomarski interference contrast optics[7] (Zeiss Universal) with ×40 water immersion objective. Condenser aperture fully opened to reduce depth of field to about 4 μm. The symbols indicate the dimensions which were measured using an ocular micrometer. The internal circumference, L, was calculated from the equation $L = (d_1+d_2)[1+(\pi/2)]+2f$. For this vessel $L_0 = 361$ μm and the segment length was 665 μm. The bar shows 50 μm. *b*, Electron micrograph of vessel shown in (*a*) sectioned radially to show the smooth muscle cells (S) and adventitia (A) in cross section. L indicates the lumen. The vessel was fixed at 36 °C at conclusion of the experiment after equilibration in relaxing solution. The vessel was prefixed on the myograph in 2.5% glutaraldehyde, 75 mM cacodylate, 4.5% sucrose, then removed from myograph and postfixed in OsO_4, 50 mM cacodylate, then blockstained in saturated aqueous uranyl acetate and embedded in Epon[19]. The bar shows 10 μm.

We used segments, about 0.7 mm long, of small (100 μm lumen) arterial resistance vessels. They were taken from the mesenteric bed of 5-month-old Wistar Kyoto rats from a point immediately proximal to the intestinal wall. Each vessel segment was threaded on to two wires attached respectively to a force transducer and a displacement device[8] (Fig. 1). This arrangement enabled the wall tension P and vessel internal circumference L to be controlled. The myograph was mounted on the stage of a microscope, enabling us to measure vessel dimensions (Fig. 2*a*). Oxygenated physiological salt solutions were circulated through the test chamber at 36 °C, *p*H 7.4. The vessels were maximally activated by K^+-depolarisation in the presence of 5 mM Ca^{2+} (ref. 9) and relaxed using the same solution with Ca^{2+} replaced by 1 mM EGTA. At other times the vessels were held in Ringer solution[10]. The overall myograph compliance was less than 0.5 $\mu m\ mN^{-1}$ and has not been corrected for. We report here results obtained from four typical experiments. The vessels were all held at an internal circumference, L_0, for which their active wall tension was a maximum P_0. Results are presented as mean ± s.e. The figures all refer to the data obtained from one of these vessels.

Figure 2*a* shows the appearance of the vessels in the light microscope in the conditions described in the legend. The general outline of the smooth muscle cells was clearly seen and the dimensions of individual cells were measured. The cells were spindle shaped, of length about 65 µm and diameter 4 µm, and were orientated approximately perpendicular to the wires. In some cells internal structures such as the nuclei and mitochondria could also be identified. When the vessels were activated, changes in the appearance of the cells were followed and although there was some rearrangement of the cells there was little change in their average length. The dynamic behaviour of the smooth muscle cells was then examined by monitoring the microscope image with a television camera and recording it on videotape. This was done during quick (5 ms) changes in internal circumference imposed by the displacement device while the vessels were activated. By replaying the tape one frame at a time, the frames immediately before and after the length change (which were separated by 17 ms) could be compared. Comparisons of the two frames showed that for length changes up to at least $10\% \cdot L_0$, the length of any smooth muscle cell was always a fixed proportion of the vessel internal circumference. In other words, changes in the strain of a vessel produced the same changes in the strain of cells. Thus active force–strain data obtained from these vessels were a measure of the active characteristics of the individual smooth muscle cells.

At the conclusion of the experiments the vessels were fixed on the myograph at L_0. Electron micrographs of longitudinal sections confirmed that the structures identified in Fig. 2*a* were indeed smooth muscle cells, and that their contractile material was generally well organised and orientated parallel to the long axis of the cells. Transverse sections (Fig. 2*b*) showed that the smooth muscle cells were closely packed in one to three layers, taking up 67% of the medial layer and 20% of the total wall.

Typical mechanical data obtained from the vessels are shown in Fig. 3. The total force developed by the vessels corresponded on the basis of the light and electron microscopy to an active wall stress of 72 ± 13 mN mm^{-2}, giving an active force per smooth muscle cell of about 5 µN and an active stress for the smooth muscle cells of 360 mN mm^{-2}. The last value can be compared with the figure of 370 mN mm^{-2} obtained for smooth muscle cells in hog carotid artery[10] and at least 300 mN mm^{-2} for frog striated fibres[11]. The dynamic stiffness was measured in two ways: from the step response of the vessel (Fig. 3*b*), and from the initial portion of the response of the vessels to a quick release sufficient to discharge the tension (Fig. 3*c*). These showed that the dynamic stiffness of small displacements was $67 \pm 6\ P_0/L_0$ and $81 \pm 16\ P_0/L_0$, respectively. The complete instantaneous elastic characteristic (Fig. 3*c*) was fitted approximately by an

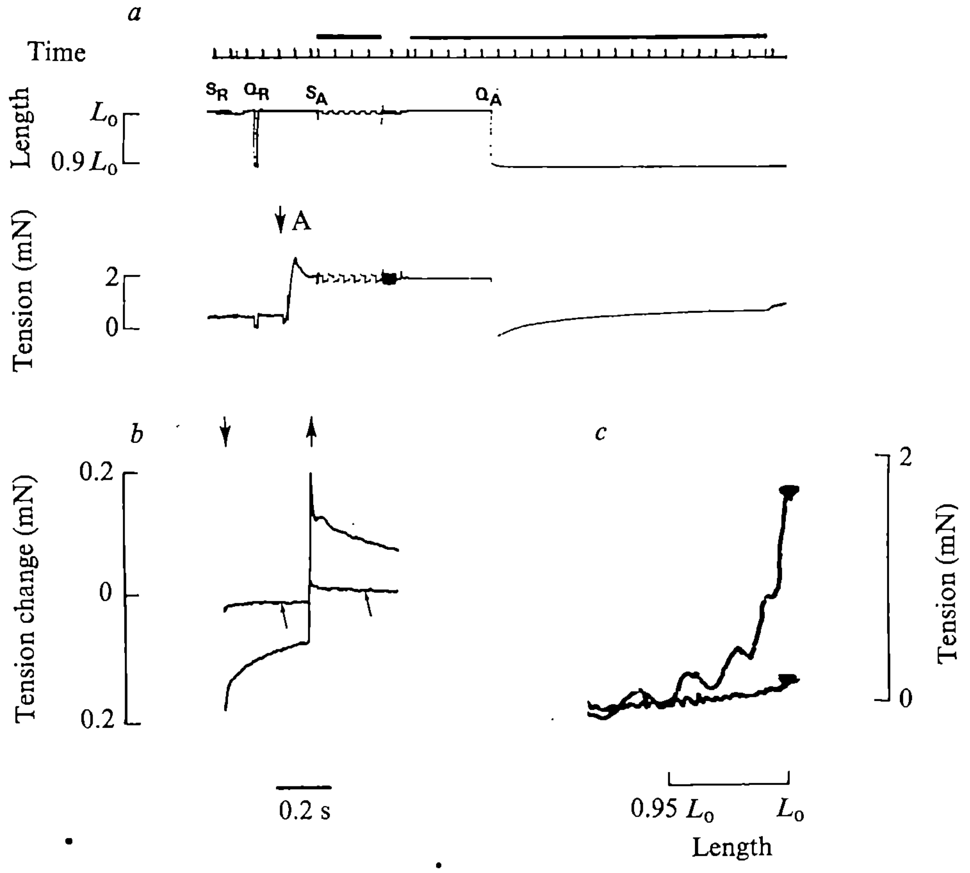

Fig. 3 Records of mechanical responses obtained from vessel shown in Figs. 1 and 2. *a*, Oscillograph record. The upper trace shows time; markers at 1-min or, under the bars (where the chart speed was increased), 1-s intervals. The centre trace shows length. The lower trace shows tension. The vessel was initially in relaxing solution (see text). At ↓A the solution was changed to activating solution. The response of the vessel to small (0.0028 L_0), repetitive (1.57 Hz) step changes in length was measured at S_R and S_A. The mean responses are shown in (*b*). The response of the vessel to quick releases (during 10 ms) through 0.086 L_0 was measured at Q_R and Q_A. The length–tension relationships during these releases are shown in (*c*). *b*, Mean of 32 tension responses to release (↓) and stretch (↑), obtained using signal averaging techniques sampling at 5-ms intervals, measured at the times indicated in (*a*) in relaxing solution (centre curves, arrowed) and in activating solution (outer curves). The active release and stretch responses, when expressed as unit step responses, $u_{-1}(t)$, are fitted approximately by the equations $u_{-1}(t) = -(P_0/L_0)(57.3-14.5 \log t)$ and $u_{-1}(t) = (P_0/L_0)(60.1-15.7 \log t)$, respectively. *c*, Oscilloscope plots of the length–tension relationship during the quick releases shown in (*a*) in relaxing solution (lower curve) and activating solution (upper curve). Vertical deflection represents tension and horizontal deflection represents length[20]. The difference between the active and relaxed responses is fitted by the equation $P = P_0 \exp[-77.6(\Delta L/L_0)]$. The oscillations on the upper curve are mechanical artefacts.

exponential equation as in hog carotid artery[12]. Analysis of the step responses (Fig. 3*b*) show that the rate constants of the tension decay and recovery (if defined as $(\mathrm{d}P/\mathrm{d}t)/(P_0-P)$) decreased throughout decay and recovery, but that their initial values were at least $0.038\pm0.005\ \mathrm{ms}^{-1}$. The corresponding figure for frog striated fibres at 0 °C is $0.4\ \mathrm{ms}^{-1}$ (ref. 13).

For the reasons discussed above, the data presented here represent the characteristics of smooth muscle cells *in situ* and, we believe, provide a basis for a better understanding of the relationship between structure and function in the smooth muscle cell. Structural studies[14,15] have shown that the contractile apparatus of smooth muscle cells is a sliding filament mechanism but that it is much less regularly organised than that of striated muscle. It is not known, however, how the contractile filaments transmit the force they generate to the extracellular environment. This contrasts with the situation for skeletal fibres, where it is clear that the contractile filaments are arranged in myofibrils which run the entire length of the fibre, being attached to the tendons at each end[16]. The dynamic stiffness of these fibres ($195\ P_0/L_0$) and their instantaneous elastic characteristic (IEC), which is approximately linear, are thought to be primarily the property of the crossbridges between the contractile filaments[17,18]. Our data for smooth muscle cells show them to have about half the dynamic stiffness of skeletal muscle fibres, while their IEC does not seem to be linear. This suggests that their dynamic compliance is divided between crossbridge compliance and a nonlinear compliance in intracellular supporting structures.

Previous mechanical studies on smooth muscle have used much larger preparations and the dynamic stiffnesses reported have been rather lower. The highest dynamic stiffness so far reported has been in hog carotid artery ($58\ P_0/L_0$)[12]. It seems likely that the differences between these results and ours may be due to a greater amount of connective tissue between the smooth muscle cells in the larger preparations. As we have shown above, in our preparation the effect of such intercellular compliance is minimal. We believe therefore that the preparation we have described may be useful in the detailed examination of the intrinsic mechanical properties of smooth muscle cells.

We thank Mr W. G. Boldosser for taking the electron micrographs and Mr Joseph P. Trono for constructing the myograph. The tungsten wire used for mounting the vessels was given by Westinghouse Corporation, Lamp Division. The work has been funded by the US National Institutes of Health.

M.J.M. is on leave-of-absence from the Biophysics Institute, Aarhus University, Aarhus, Denmark.

Note added in proof: We should like to draw attention to a recent report[21] of direct measurements of the force developed by isolated smooth muscle cells.

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Received December 15, 1975; accepted February 17, 1976.

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Calcium activation produces a characteristic response to stretch in both skeletal and cardiac muscle

RESTING frog skeletal muscle, when stretched at a constant velocity, exhibits a force response which, for length changes less than about 0.2%, is elastic in nature. First observed by Hill[1], this response was attributed to a short range elastic component (SREC) that was thought to be a property of a small number of cross bridges which, even in the resting state, extend from the myosin-containing thick filaments to interact with sites on the actin-containing thin filaments. This view was criticised[2] on the grounds that the SREC stiffness remained approximately constant as the amount of thick and thin filament overlap was decreased by increasing muscle length. On the basis of the sliding filament theory[3,4], SREC stiffness would be expected to decrease because reduced amounts of thick and thin filament overlap would result in a decreased number of cross bridges available in interact with thin filament sites. Flitney and Hirst[5] have reported that in twitch contractions of frog skeletal muscle a short range elastic response is present and is similar in form to that of resting muscle. In this case, however, a directly proportional relationship was found between the short range stiffness and the amount of overlap between the thick and thin filaments, which suggests that this stiffness is a property of attached cross bridges. To investigate the possible presence of attached cross bridges in resting muscle, we have measured the calcium dependence of the short range stiffness in skeletal and cardiac muscle preparations in which the myofibrils were directly exposed to the bathing medium.

Single muscle fibres were isolated from the semitendinosus muscle of the frog (*R. pipiens*) and their sarcolemmae were

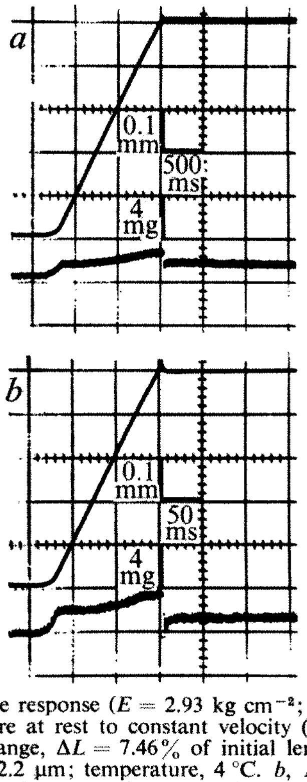

Fig. 1 *a*, Force response ($E = 2.93\ \mathrm{kg\ cm^{-2}}$; lower) of living frog skeletal fibre at rest to constant velocity (0.06 ML per s; total length change, $\Delta L = 7.46\%$ of initial length, L_i) stretch (upper). SL = 2.2 μm; temperature, 4 °C. *b*, As for *a* except velocity is 0.60 ML per s and $E = 5.70\ \mathrm{kg\ cm^{-2}}$. Responses are typical, demonstrating a steep initial rise which is the elastic part of the response, reaching an elastic limit at a change in length of about 0.3% and followed by Hill's[1] frictional phase as the stretch is continued. Short range elastic stiffness (expressed as modulus, E) is calculated as the height of the initial, fast part of the force response divided by the corresponding length change.

either removed by mechanical skinning[5] or made highly permeable by chemical treatment (that is, chemical skinning) with glycerine and a non-ionic detergent[6]. Thin longitudinal strips were cut from rabbit right ventricular papillary muscles and placed in glycerinating solution[6] at 0–5 °C for 3 d. Muscle segments, 1–3 mm long, were attached to wires extending from a force transducer and servo motor by means of glass jaws—two pieces of glass coverslip held flat together by slightly sprung strips cut from a stainless steel razor blade. This connection proved to be isometric, since no muscle movement with respect to the jaws was observed through the light microscope at ×400 magnification. Constant velocity length increases were applied at one end of the muscle preparation through an arm attached to the servo motor. Force was measured using a capacitance transducer with a sensitivity of 1 mV mg^{-1} and a natural resonance frequency of 800 Hz. All measurements were made at 4 °C. The solutions, apparatus and methods for controlling free Ca ion concentration using EGTA have been described[6]. Sarcomere length (SL) was measured and monitored continuously using a light microscope[6].

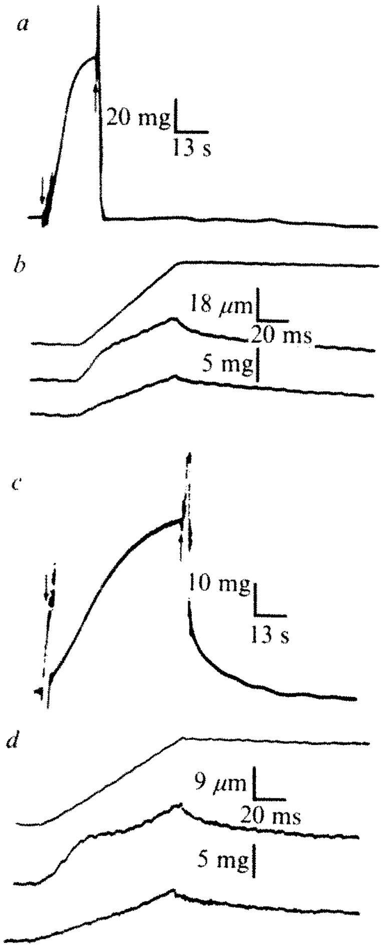

Fig. 2 *a*, Contraction time-course of glycerinated skeletal fibre when activating solution is applied (pCa = 5.76; first arrow) followed by relaxing solution (pCa about 9; second arrow). SL = 2.28 μm. *b*, Force responses of glycerinated skeletal fibre to constant velocity (0.34 ML per s; total ΔL = 1.90% L_i) stretch (upper) in relaxing solution (pCa about 9; E = 4.55 kg cm^{-2}; lower) and activating solution (pCa = 6.51; E = 15.3 kg cm^{-2}; middle). SL = 2.28 μm. *c*, Contraction time course of glycerinated papillary muscle strip when activating solution is applied (pCa = 5.76; first arrow) followed by relaxing solution (pCa about 9; second arrow). SL = 2.14 μm. The slower time course relative to that of the skeletal fibres is typical of the heart preparations used. *d*, Force responses of glycerinated papillary muscle strip to constant velocity (0.46 ML per s; total ΔL = 3.66% L_i) stretch (upper) in relaxing solution (pCa about 9; E = 2.25 kg cm^{-2}; lower) and activating solution (pCa = 6.51; E = 6.75 kg cm^{-2}; middle). SL = 2.14 μm.

The calcium sensitivity of the three preparations was quantified to establish a basis for comparison of the stiffness measurements which follow. In each case, the calcium threshold for active tension development was near a pCa (negative log of free Ca ion concentration) of 6.9. At higher calcium ion concentrations the typical S-shaped relationship between tension and pCa was confirmed[6]; in all cases no further increases in active tension development were noted at pCa less than 6.

Short range elastic stiffness was measured first on living, resting single frog skeletal muscle fibres (Fig. 1). At a velocity of stretch of 0.06 muscle lengths (ML) per second, an elastic modulus (E) of 2.93 kg cm^{-2} was measured, comparing well with the value of 2.23 kg cm^{-2} found by Lannergren[7] using a slower rate of stretch (about 0.005 ML per s) and a similar preparation. In accordance with the findings of Hill[1], we found that the measured value of E rose as a function of increasing velocity of stretch, an effect especially prominent at velocities greater than 0.004 ML per s. A stretch rate of around 0.40 ML per s was chosen for use in the stiffness measurements involving the chemically activated preparations in order to take advantage of the enhancement of the response at higher stretch velocities.

Constant velocity stretches were applied to the mechanically and chemically skinned muscle preparation (Fig. 2) while in relaxing solution, in which there is no added cal-caffeine. The nearly exact similarity of the tension responses of the type reported by Hill for living muscle, and shown in Fig. 1, was found. Instead, tension increased steadily with increasing length to a level characteristic of the final length, there being some slight stress-relaxation when movement stopped. The form of the response changed considerably, to that typical of living muscle, when solutions containing small amounts of added calcium were applied. The threshold for this transformation was about pCa 7. At pCa less than 7, the measured short range stiffness increased sharply with decreasing pCa. No stiffness measurements were made, however, at pCa less than 6.09, because marked decreases in active tension development during subsequent contractions were observed after stretches at these pCa levels.

We investigated the possibility that the sarcoplasmic reticulum (SR) has a role in determining the tension response of muscle to constant velocity stretch. Caffeine (10 mM) in the presence of 0.1 mM EGTA was applied to each preparation after successive treatments with a normal activating solution (pCa 6.7) and relaxing solution containing low EGTA (0.1 mM), a procedure similar to that described by Endo[8]. Only the mechanically skinned skeletal fibres showed a functionally intact SR, as judged by a large tension response of transient duration after application of caffeine. The nearly exact similarity of the tension responses to stretch in the mechanically and chemically skinned fibres indicates that the SR does not have a major influence in the determination of short range stiffness.

These results show that the short range stiffness of resting muscle depends on the concentration of calcium in the fluid bathing the myofibrils. Calcium is believed to trigger the interaction of cross bridges with sites on the thin filaments by binding with the regulatory protein troponin which is located at intervals along the thin filaments[9]. Also, X-ray diffraction data suggest that in skeletal muscle activation may affect cross-bridge configuration causing the cross bridges to move out from the thick filaments and toward the thin filaments[10]. Other X-ray work[11] indicates that in living, isolated heart muscle a few cross bridges may be in an activated, though not necessarily attached, position while the muscle is at rest. Therefore, the short range stiffness seems to be a property of calcium-activated cross bridges which have either become attached to thin filament sites or at least changed from a resting to an activated position. To this extent, our work confirms Hill's hypothesis[1] that cross bridges are the source of the SREC.

The finding that the short range elastic response is virtually absent in muscles bathed in relaxing solution but is present at *p*Ca near 7 implies that the calcium concentration in the myoplasm of living fibres at rest is about *p*Ca 7. Finally, the short range elasticity has for the first time been shown to be present in a heart muscle preparation. The discovery of this elastic response as a characteristic of both heart and skeletal muscles suggests that the calcium mechanism which underlies the response is the same in the two cases.

This work was supported by grants from the National Institutes of Health, the US National Science Foundation, the Muscular Dystrophy Association of America, the American Heart Association and the Massachusetts Heart Association, and by a National Institutes of Health postdoctoral fellowship to R.L.M.

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Received December 24, 1975; accepted February 25, 1976.

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Ultrastructural identification of granules containing oxytocin and vasopressin

OXYTOCIN and vasopressin, the hormones of the mammalian hypothalamo–neurohypophysial complex, are synthesised in the supraoptic (SON) and paraventricular (PVN) nuclei and are secreted into the blood stream from the neurohypophysis. It is now thought that the two hormones are produced in both nuclei, but are stored, together with their corresponding neurophysins, in distinct neurosecretory granules located in separate perikarya and axons, on the basis of data from various species including fish, reptiles, birds and mammals[1–4]. This study describes cytochemical experiments carried out on tissue from normal rats and from rats homozygous for hereditary diabetes insipidus (Brattleboro strain) that support this view and allow a tentative identification of the two granule types.

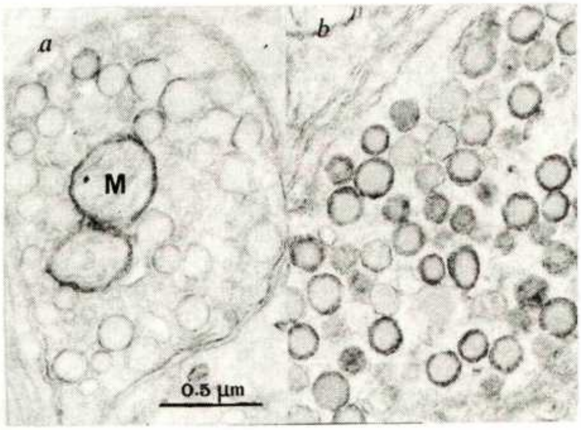

Fig. 1 Secretory axons in the neurohypophysis of normal rats. Potassium ferrocyanide–OsO_4 method. *a*, A neurosecretory axon with non-reactive granules. *b*, An axon in which the granules have the ring-shaped electron-dense deposit around their core. M = mitochondria.

Three methods believed to detect glycoproteins at the ultrastructural level were used: the periodic acid–thiocarbohydrazide–silver proteinate technique[5], the periodic acid–chromic acid–silver methenamine technique[6] and the potassium ferrocyanide–osmium technique[7]. Following each reaction, two distinct types of neurosecretory axon were recognised within the neural lobes of normal rats (Sprague–Dawley strain)[8,9]. In one type of axon, most granules had a ring-shaped silver or ferrocyanide–osmium deposit around their core. In the other type of axon, all granules had an homogeneous content of low electron density (Fig. 1). This contrasting aspect could be enhanced further by combining uranyl acetate impregnation *en bloc* with a subsequent proteinate silver reaction on ultrathin sections. Figure 2 shows the characteristic, positive reaction of the granules in one axon from a neural lobe treated with uranyl acetate *en bloc*. Variations in the plane of section may account for the unequal intensity of the reaction in granules of the positive axon. A small number of unreactive granules were sometimes seen intermingled among reactive granules, but they usually had an electron-lucent space between the membrane and core which was absent in the granules seen in non-reactive axons. Of 3,400 axons in normal Sprague–Dawley neurohypophyses, 68% were found to be positive. Axons of each type frequently occurred in small groups.

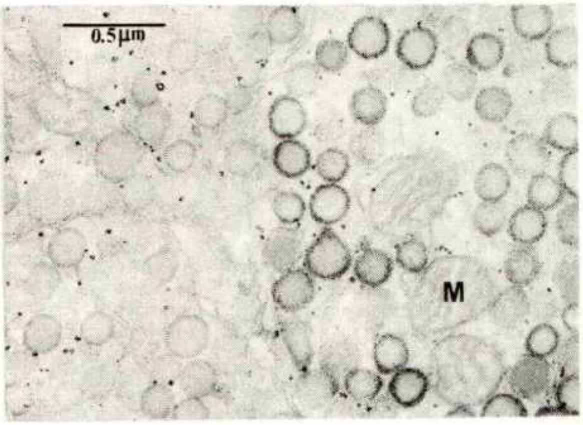

Fig. 2 Secretory axons in the neural lobe of a normal rat. Uranyl acetate and periodic acid–thiocarbohydrazide–silver proteinate method. Note presence of axons containing non-reactive granules (left), and reactive granules in another, adjacent axon (right).

The same methods revealed a similar duality in the hypothalamic magnocellular nuclei. This was particularly obvious in PVN, where perikarya and axons contained either reactive granules or non-reactive ones. In SON, the non-reactive type seemed to be less frequent, at least in the portion lateral to the optic chiasma, the region usually investigated for technical convenience.

These results indicate, therefore, that a distinction can be made between two types of neurosecretory axons and perikarya in normal rats. Since homozygous Brattleboro rats are characterised by an absolute deficiency in vasopressin (and vasopressin–neurophysin) biosynthesis[10,11], the same methods were applied to 19 of these animals to identify those axons and perikarya containing oxytocin granules. Before fixation, 9 animals were treated for 1 month with exogenous vasopressin (Pitressin tannate, Parke–Davis, 1 IU d^{-1} subcutaneously)[12]. We found that granulated axons were more frequent in the neurohypophysis of treated rats. They generally occurred in clusters surrounded by areas devoid of neurosecretory granules. All the granules observed in the neural lobes of homozygous Brattleboro rats were non-reactive (Fig. 3*a*). There was some deposit of silver proteinate on the granule-limiting membranes, in particular following treatment with uranyl

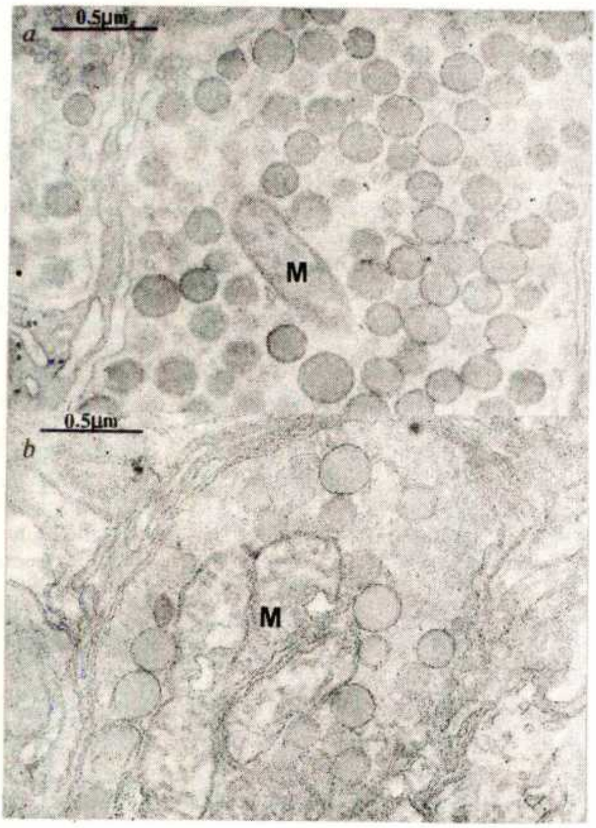

Fig. 3 Micrographs from homozygous Brattleboro rats treated with Pitressin tannate. Periodic acid–thiocarbohydrazide–silver proteinate method. Note that in the neurohypophysis (*a*) and in the hypothalamic paraventricular area (*b*) secretory axons contain only axons of the non-reactive type.

acetate *en bloc*, but the granule cores appeared homogeneous and did not show the characteristic annulate density. As in normal rats, we also studied the PVN and that part of the SON which is adjacent to the optic chiasma. The granules found in the axons and perikarya of these nuclei in homozygous Brattleboro rats were also non-reactive (Fig. 3*b*). Normal rats of the same strain (Long–Evans) served as controls. Of 1,600 neurohypophysial axons examined, 34% contained the reactive granules.

In view of the absence of reactive granules in homozygous Brattleboro rats, we propose that the reactivity of the neurosecretory material to the histochemical 'stains' used in this study is associated with the vasopressin-containing granules. Oxytocin is thus stored in the non-reactive granules. We do not at present know what accounts for the reactivity of the vasopressin granules, but their difference from the oxytocin granules may be in glycoprotein composition. Our results also support the idea that vasopressin and oxytocin are present in distinct neurones found in both PVN and SON.

We thank Mrs S. Rua for technical assistance, the Institut für medizinischbiologische Forschung, Fillensdorf, for supplying the Brattleboro rats, and the CNRS and INSERM for support.

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Primary structure of human β-lipotropin

AFTER the discovery and isolation of β-lipotropin from sheep pituitary glands[1,2], the hormone has also been obtained in highly purified form from bovine[3], porcine[4–6], and human[7,8] pituitaries. The complete amino acid sequences of ovine[9,10] and porcine[11–13] hormones have been proposed. To gain insight into the evolutionary history of lipotropins and their structure–function relationships, and in view of recent data on the opiate agonist activity of peptide fragments from β-lipotropin[14,15], we report here the complete amino acid sequence of the human hormone.

Highly purified β-lipotropin (β_h-LPH) was obtained from fresh-frozen human glands by the procedure previously described[9] for the ovine hormone with minor modifications. It behaved as a single component in disc electrophoresis and partition chromatography experiments. Sedimentation equilibrium data gave a molecular weight of 11,700. Amino acid analysis, performed in the automatic amino acid analyser by the procedure of Moore[16] gave the following empirical formula (91 amino acid residues): Trp (1) Lys (9) His (2) Arg (5) Asp (10) Thr (4) Ser (4) Glu (11) Pro (6) Gly (11) Ala (8) Val (2) Met (2) Ile (2) Leu (8) Tyr (3) Phe (3). The NH_2-terminal sequence as determined by the dansyl–Edman procedure[17,18] was found to be: Glx–Leu–Thr–Gly–Glx–Arg–Leu–Arg–.

For the primary structure determination, a tryptic digest of β_h-LPH (3 mg) was fractionated by the two-dimensional paper chromatography–electrophoresis as described previously[19]. The eluates from each spot were submitted to amino acid[16] and sequence[17,18] analyses. Amino acid contents of the twelve tryptic peptides account for the composition of the hormone. All tryptic peptides have been completely sequenced except T12 which has 31 amino acids with the following composition: Lys (1) His (1) Asp (6) Ser (1) Glu (4) Pro (2) Gly (5) Ala (6) Val (1) Leu (4).

In order to obtain the sequence of T12, it was necessary to isolate the peptide by submitting the tryptic digest of β_h-LPH (17 mg) on Sephadex G-25 (fine) in 0.1 M HOAc. The material in the second peak was recovered by lyophilisation and shown to be peptide T12 with a yield of 3.3 mg. Two samples of T12 (1 mg) were digested separately by pepsin and thermolysin; the digests were mapped and eluates of each spot were subjected to amino acid and sequence analyses. For the determinations of Asp/Asn and Glu/Gln, amino acid analysis of the leucineaminopeptidase and electrophoretic mobility of *p*H 6.7 on paper were used.

The following chymotryptic peptides (see Fig. 1) were obtained by two dimensional chromatography-electrophoresis of the chymotryptic digest (1 mg) in order to align the tryptic peptides: C17B, C8, C18, C23, C4, C16, C5, C15, and C2. These chymotryptic peptides were analysed for amino acid composition and partially sequenced. From these data, the complete amino acid sequence of β_h-LPH is proposed as shown in Fig. 1.

The sequence of COOH-terminal 52 amino acid residues

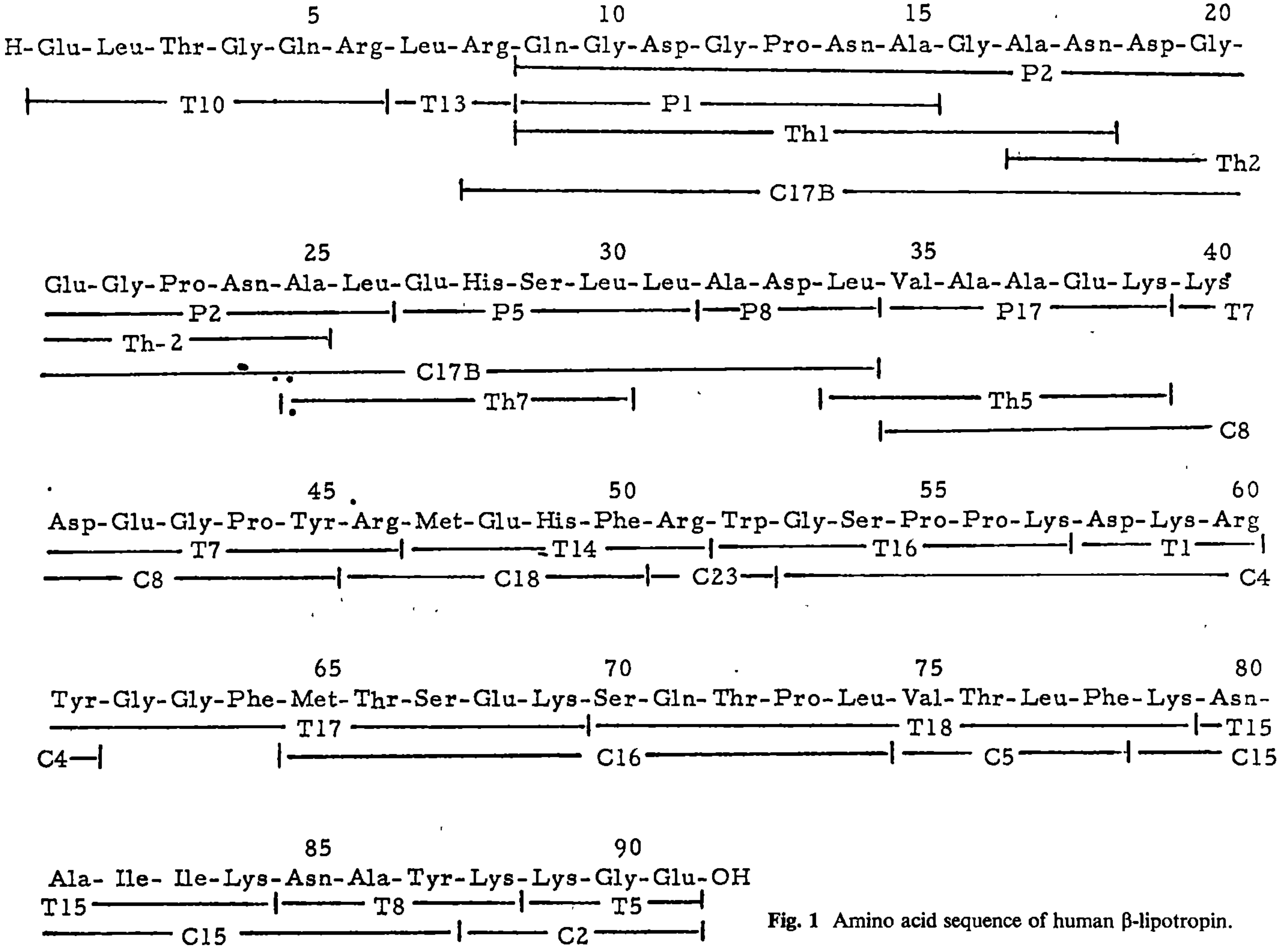
5 10 15 20
H-Glu-Leu-Thr-Gly-Gln-Arg-Leu-Arg-Gln-Gly-Asp-Gly-Pro-Asn-Ala-Gly-Ala-Asn-Asp-Gly-
P2
T10
T13
P1
Th1
Th2
C17B
25 30 35 40
Glu-Gly-Pro-Asn-Ala-Leu-Glu-His-Ser-Leu-Leu-Ala-Asp-Leu-Val-Ala-Ala-Glu-Lys-Lys
P2
P5
P8
P17
T7
Th-2
C17B
Th7
Th5
C8
45 50 55 60
Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-Lys-Arg
T7
T14
T16
T1
C8
C18
C23
C4
65 70 75 80
Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
T17
T18
T15
C4
C16
C5
C15
85 90
Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
T15
T8
T5
C15
C2

Fig. 1 Amino acid sequence of human β-lipotropin.

has been reported by Cseh *et al.*[20]. In addition, these investigators indicated that their β_h-LPH preparation has the NH_2-terminal sequence: NH_2–Glx–Gly–Asx–. Our data, shown in Fig. 1, are in agreement with the Hungarian workers on the COOH-terminal sequence but differed on the sequence at the NH_2-terminus.

From a comparison of the proposed structure of β_h-LPH with the lipotropin sequences of other species[9–12], it is evident that the sequence of COOH-terminal 56 residues is surprisingly homologous whereas the amino acid sequence at the NH_2-terminus exhibits considerable variability as shown in Fig. 2.

Fig. 2 Comparison of the amino acid sequence of β_h-LPH with that of the ovine and porcine hormone.

	5 10	15 20
Human:	H-Glu-Leu-Thr-Gly-Gln-Arg-Leu-Arg-Gln-Gly-	Asp-Gly-Pro-Asn-Ala-Gly-Ala-Asn-Asp-Gly-
Ovine:	H-Glu-Leu-Thr-Gly-Glu-Arg-Leu-Glu-Gln-Ala-	Arg-Gly-Pro-Glu-Ala-Gln-Ala-Glu-Ser-Ala-
Porcine:	H-Glu-Leu-Ala-Gly-Ala-Pro-Pro-Glu-Pro-Ala-	Arg-Asp-Pro-Glu-Ala-Pro-Ala-Glu-Gly-Ala-

25 30	35 40
Glu-Gly-Pro-Asn-Ala-Leu-Glu-His-Ser-Leu-	Leu-Ala-Asp-Leu-Val-Ala-Ala-Glu-Lys-Lys-
Ala-Ala-Arg-Ala-Glu-Leu-Glu-Tyr-Gly-Leu-	Val-Ala-Glu-Ala-Glu-Ala-Ala-Glu-Lys-Lys-
Ala-Ala-Arg-Ala-Glu-Leu-Glu-His-Gly-Leu-	Val-Ala-Glu-Ala-Gln-Ala-Ala-Glu-Lys-Lys-

45 50	55 60
Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-	Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-Lys-Arg-
Asp-Ser-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-	Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-Lys-Arg-
Asp-Glu-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-	Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-Lys-Arg-

65 70	75 80
Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-	Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-	Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-	Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-

85 91
Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
Ala-Ile-Ile-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln-OH
Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln-OH

We thank Jean Knorr and Kenway Hoey for technical assistance. This work was supported in part by the US National Institutes of Health.

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Cross tolerance between morphine and methionine-enkephalin

TOLERANCE and dependence are characteristic symptoms in man during prolonged use of opiates. We have pointed out[1] that for an understanding of these phenomena the interaction between endogenous enkephalin and exogenous opiates is of importance. Normally, enkephalin can be assumed to control inhibitory mechanisms determining the rate of neurotransmitter release. If, however, opiates are administered with the intent of increasing the effects of these inhibitory mechanisms, then control will pass from the endogenous enkephalin to the exogenous opiates. Since tachyphylaxis is a pharmacological characteristic of opiates, a state of tolerance will arise in which increasing amounts of opiates will be required to maintain the desired effect. As an important step in the elucidation of the mechanism of action of enkephalin, it was necessary to establish whether cross tolerance between enkephalin and opiates is found in morphine-tolerant animals. This point was tested in two morphine-sensitive models, the myenteric plexus–longitudinal muscle preparation from the guinea pig ileum[2,3] and the mouse vas deferens[4]. As natural enkephalin consists of about 75% methionine–enkephalin and 25% leucine–enkephalin[5], the relative potencies of synthetic methionine–enkephalin and morphine were compared in preparations obtained from animals with different degrees of tolerance. Cross tolerance between morphine and methionine–enkephalin was shown to occur in the two preparations obtained from animals implanted with morphine pellets.

Mice were implanted with 1 or 2 pellets, each containing 75 mg morphine base, and guinea pigs with 2 or 4 pellets. After 3 d the animals were killed; the myenteric plexus longitudinal muscle[2] was prepared for isometric recording or the vas deferens[4] for isotonic recording. The bathing medium contained either no morphine or, to imitate the *in vivo* conditions, 0.5, 1 or 2 μM morphine. After approximately 2 h of exposure, the morphine was washed out and, when the sensitivity to morphine or methionine–enkephalin stopped increasing, dose–response curves for morphine and methionine–enkephalin were obtained and the ID_{50} values calculated. Preincubation with morphine had no consistent effect on the ID_{50} values.

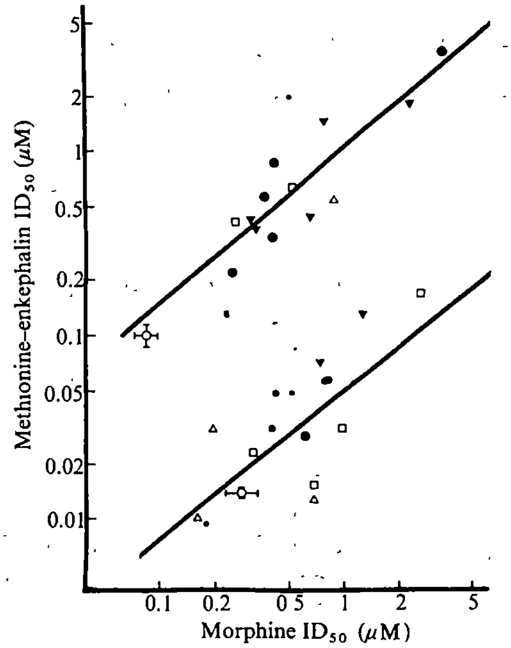

Fig. 1 Correlation between ID_{50} values of morphine and methionine–enkephalin. Myenteric plexus–longitudinal muscle preparation of the guinea pig ileum (upper line) and mouse vas deferens (lower line) from controls and animals implanted with pellets of morphine base. Controls, ○; implanted animals, tissues not preincubated with morphine, □; implanted animals, tissues preincubated with morphine, △ 0.5 μM, ▼ 1 μM, ● 2 μM. The lines were drawn from calculated regressions, the slope of the upper line being $b = 0.84$ and that of the lower line $b = 0.79$.

The results (Fig. 1) show that in both preparations tolerance of varying degree had developed. In the ileum from control guinea pigs the ID_{50} of morphine was 94.4 ± 13.2 nM and that of methionine–enkephalin 114.7 ± 19.7 nM, with a potency ratio of enkephalin to morphine of 0.89 ± 0.08 ($n = 12$). In the preparations from pellet-implanted guinea pigs, the ID_{50} values were increased 3 to 400-fold but the mean potency ratio of 0.97 ± 0.10 ($n = 13$) did not differ from that found in the controls. Similar results were obtained for the mouse vas deferens, although the correlation was not as good as in the myenteric plexus and there was an overlap of the ID_{50} values of preparations from pellet-implanted and non-implanted mice. The potency ratio of enkephalin to morphine was 21.5 ± 4.6 ($n = 4$) in the controls and 22.9 ± 5.3 ($n = 10$) in preparations from pellet-implanted mice.

The molecular mechanisms underlying cross tolerance are uncertain. Since the number of receptor sites in brain or their affinity to opiates are the same in normal and morphine-tolerant rats[6], tolerance and, therefore, also cross tolerance may be due to a process subsequent to the activation of the recognition sites of the receptor complex. This possibility is supported by the observation that the myenteric plexus–longitudinal muscle preparation obtained from morphine-tolerant guinea pigs shows reduced sensitivity to the depressant effects not only of morphine but also of adrenaline and noradrenaline[7,8]. The importance of this observation lies in the fact that the depressant effect of catecholamines is due to activation of presynaptic α-adrenoceptors[9] which are different and separate from opiate receptors[10]. It is possible that changes in adenylate cyclase activity[11,12] represent the common factor in the simultaneous changes in the sensitivity to opiates and catecholamines.

This work was supported by grants (to H.W.K.) from the US National Institute on Drug Abuse, the Medical Research Council and the US Committee on Problems of Drug Dependence, NAS-NRC. We thank Dr B. A. Morgan (Reckitt and Colman) for synthetic methionine-enkephalin.

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Received February 4; accepted February 24, 1976.

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Analgesia induced *in vivo* by central administration of enkephalin in rat

ENKEPHALIN, a natural opiate receptor agonist in brain[1–4], has been identified by Hughes *et al.*[5] as a mixture of two pentapeptides: H–Tyr–Gly–Gly–Phe–Met–OH (methionine–enkephalin) and H–Tyr–Gly–Gly–Phe–Leu–OH (leucine–enkephalin). Both peptides mimic the ability of morphine to block electrically evoked contractions of mouse vas deferens and guinea pig ileum, and both inhibit the stereospecific receptor binding of the opiate antagonist ^{3}H-naloxone in brain homogenates[5]. On the basis of this and other[6–8] evidence, it has been proposed[2,9,10] that enkephalin receptors may be sites at which morphine-like drugs exert their analgesic actions, and that the enkephalins themselves may be modulators or transmitters in brain systems for pain suppression or analgesia. Consistently with this suggestion, we find that methionine–enkephalin and leucine–enkephalin, when administered through permanently indwelling cannulae in the lateral ventricles of rats, induce a profound analgesia *in vivo* that is fully reversible by naloxone.

Methionine–enkephalin and leucine–enkephalin were synthesised by the solid phase method. After purification by gel filtration, homogeneity of the peptides was verified by amino acid analysis and thin-layer chromatography. Intraventricular cannulae, constructed from 27-gauge stainless steel tubing, were implanted in 300–400-g male Charles River rats under pentobarbital anaesthesia, as described previously[11]. After a recovery period of at least one week, the rats were tested in the tail-flick procedure of D'Amour and Smith[12], an analgesic test with good selectivity for opiate drugs[13]. Animals were comfortably restrained in a wire-mesh cylindrical chamber so that the tip of the tail could be positioned at the focal point of a radiant heat source (1183 GE lamp). After an acclimatisation period of 15 min, the lamp was turned on and an electric clock was activated. Movement of the tip of the tail exposed a photocell to the lamp, which turned off the thermal stimulus and stopped the clock. If the rat failed to move its tail within 8 s, the heat was automatically turned off to prevent blistering. The intensity of the lamp was adjusted in each case to produce at least three baseline tail-flick latencies of 2.5–5 s. Tests were made every 2 min.

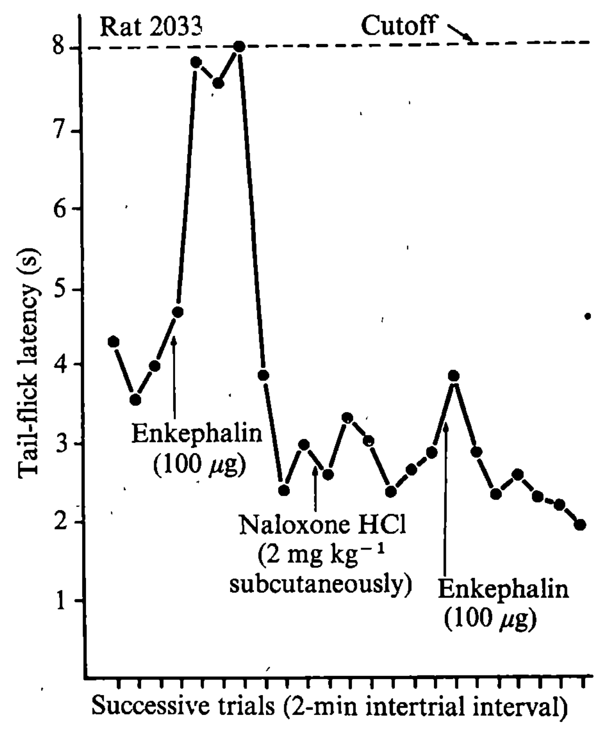

Fig. 1 Analgesic effect of intraventricularly-administered methionine–enkephalin and its prevention by subcutaneous administration of naloxone in a representative rat. Note especially the brief delay in onset of the first enkephalin response, and the apparent absence of effect on response latency of naloxone itself.

After preliminary experiments to establish the effective dose range, 100 or 200 μg of methionine–enkephalin or leucine–enkephalin was dissolved in 10 μl of Ringer's solution and injected in the lateral ventricle at a rate of 0.2 μl s^{-1}. Injections of Ringer's solution at *p*H 4.0 (to approximate the *p*H of the enkephalin solutions) served as control. To minimise disturbance of the rats, connections to the intraventricular cannula were made before the baseline tests. Behavioural reactions to the injection itself were rarely observed.

Statistically significant increases in tail-flick latency

Table 1 Analgesic activity of enkephalin and morphine as assessed by an increase in the latency of a tail-flick response to noxious thermal stimulation in different groups of rats

Drug	Dose (μg)	No. of rats	Mean latency±s.e.m. Baseline	Mean latency±s.e.m. Drug	Mean % change±s.e.m.
Ringer's (*p*H 7.4)		10	3.73±0.27	3.94±0.25	7.4±6.2
Ringer's (*p*H 4.0)		13	4.02±0.20	4.27±0.28	6.6±6.9
Morphine sulphate	10	7	3.96±0.42	6.59±0.73†	65.4±9.1
Methionine–enkephalin	100	24	3.83±0.14	5.19±0.31†	37.2±8.8
	200	3	2.88±0.41	4.99±0.85*	77.8±35.8
Leucine–enkephalin	100	11	3.97±0.17	5.11±0.34*	31.7±11.5
	200	4	3.35±0.35	5.54±0.72†	70.5±29.3

*Drug minus baseline significantly different from Ringer's (*p*H 4.0) minus baseline, $P < 0.05$.
†$P < 0.01$.
All drugs were dissolved in 10 μl of Ringer's solution and injected in the lateral ventricle through permanently-indwelling cannulae. Numbers give mean latencies of three "baseline" and five "drug" responses recorded consecutively at 2-min intervals just before and after injections.

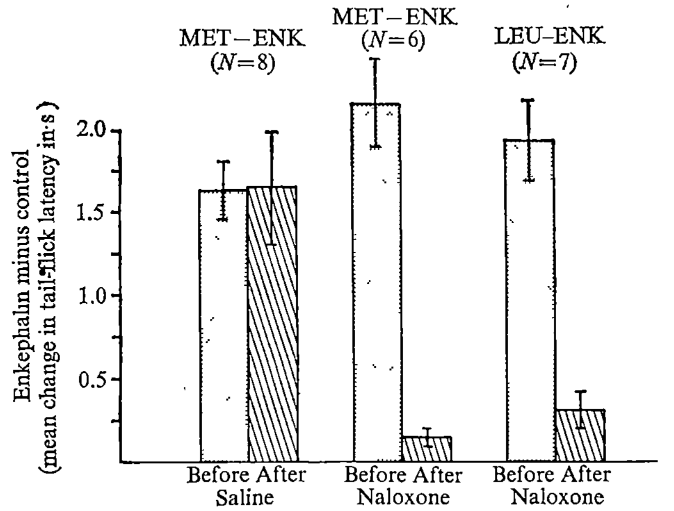

Fig. 2 Summary data of naloxone-reversal experiments. Methionine-enkephalin (MET–ENK) or leucine–enkephalin (LEU–ENK), in intraventricular doses of 100 or 200 μg, was administered both before (stippled) and 12–16 min after (hatched) subcutaneous injections of 2 mg kg^{-1} of naloxone (or saline). Analgesic responses to both peptides were significantly ($P < 0.025$) reduced following naloxone. Bars indicate standard errors.

followed injections of both peptides (Table 1); in most cases, the 8-s cutoff was reached at the peak of the drug action. The analgesic effect developed rapidly after a definite latent period (2–6 min) and dissipated rapidly (10–12 min) (Fig. 1). Although systematic dose–effect data were not obtained, the larger dose of both substances produced a stronger effect. Methionine–enkephalin and leucine–enkephalin were similar in potency, but each was less potent than morphine by an order of magnitude or more. The analgesia caused by intraventricular administration of 10 μg of morphine sulphate matched in intensity and reliability that induced by 200 μg of the enkephalins, although the effects of morphine lasted substantially longer (>1 h). The weaker potency and shorter duration of action of the natural substances may be attributed at least in part to their relatively rapid degradation by brain enzymes[2]. Slow permeation of the peptides to deeply situated receptor sites, as suggested by the delay in onset of action, would further favour their inactivation.

Blockade of the analgesic response by naloxone was evaluated in 13 rats that had exhibited a reliable effect to either methionine–enkephalin or leucine–enkephalin. After the effects of the first dose of enkephalin had clearly worn off, 2 mg kg^{-1} of the opiate antagonist was administered subcutaneously. 12–16 min later, at the approximate peak of the action of naloxone, the enkephalin dosing was repeated. In all naloxone-treated rats, the analgesic response to the second injection of peptide was virtually abolished (Fig. 2). In control experiments, subcutaneous administration of saline in place of naloxone was shown to have no measurable effect on the response to methionine–enkephalin.

Our observations suggest that enkephalin has opiate-like analgesic properties. In pharmacological studies of pain perception, however, especially when a substance is injected directly in the brain, serious consideration must be given to the possibility of artefacts. This possibility seems remote in the present case for several reasons. First, the detailed pattern of analgesic activity after systemic administration of morphine is reproduced by microinjections of the drug into the ventricles or central grey matter of the brain[7,8,14]. Second, as already indicated, the D'Amour–Smith test is highly selective for opiate-like activity and tends to yield negative results for other drugs, even non-opiate analgesics. Finally, naloxone blockade may be taken as a specific indication that enkephalin induces analgesia by activation of opiate receptors. We therefore conclude that our findings lend support to the concepts of Hughes[2] and Liebeskind et al.[7] that enkephalin may serve as a natural opiate-like neurohormone in a central mechanism for the inhibition of pain.

We thank Herman Morris and William Carmint for technical assistance.

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Received February 9; accepted February 29, 1976.

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Inability of bats to synthesise L-ascorbic acid

RECENT speculations on the loss and retention of the ability to synthesise L-ascorbic acid (vitamin C) during evolution of animals have been based on few data, often for limited numbers of species[1]. Published reports for the Mammalia deal mostly with domesticated and laboratory species, and there is little information on ascorbate biosynthesis in wild populations. The status of knowledge on bats typifies this paucity of data. It has been reported that two species of bats (of approximately 850 species), like anthropoid primates and guinea pigs, are unable to synthesise vitamin C because they lack L-gulonolactone oxidase[2]. When present, this enzyme catalyses the final step in the biosynthesis of L-ascorbic acid from glucose. These species are *Pteropus medius*[3] (=*P. giganteus*), a fructivore of the suborder Megachiroptera, and *Vesperugo abramus*[2,4] (presumably *Pipistrellus coromandra*), an insectivore of the only other chiropteran suborder, the Microchiroptera. We have assayed livers of 34 species of New World microchiropteran bats for L-gulonolactone oxidase (Table 1). These species represent six families, temperate and tropical environments, and a wide variety of food habits and life histories. Within the limits of our assay, we could not detect L-gulonolactone oxidase in livers of any of the species examined. This greatly strengthens the hypothesis that all members of the Chiroptera lack the ability to synthesise vitamin C, which is surprising considering the diversity of bats and their many highly specialised diets.

L-Gulonolactone oxidase has been detected in the microsomal fraction of liver cells[5] in all mammals shown to synthesise ascorbate. Most previously reported investigations involved a technique in which microsomes are prepared from liver homogenate by differential centrifugation, and only the microsomal fraction is analysed for the enzyme[5]. We have shown, however, that a large portion of the enzyme may remain with other fractions and thus not be recovered with the microsomes[6]. Enzyme activities for *Pteropus* and *Vesperugo* were reported[3,4] as "nil".

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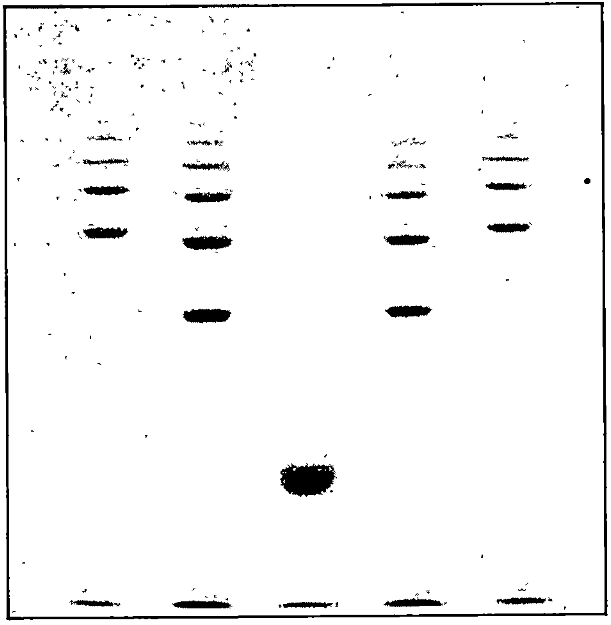

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Although these workers probably used a quantity of microsomal preparation equivalent to 0.25 g of liver in the assay, the sensitivity of their method cannot be assessed adequately because of the potential for loss of any existing L-gulonolactone oxidase during preparation of the microsomes.

A more sensitive method[6], in which essentially all the L-gulonolactone oxidase is solubilised by homogenising liver tissue in a medium containing sodium deoxycholate, was used for the assays reported here. This method detects lower levels of L-gulonolactone oxidase in liver than does the microsomal method. When assays are conducted on individuals the techinique may be limited by the small size

Table 1 Bats examined for L-gulonolactone oxidase in the liver

Species examined	No. and sex of specimens ♂♂	♀♀	Specimen origin*	L-Gulonolactone oxidase activity†
Family Noctilionidae				
Noctilio leporinus	2		Tropics	< (0.06–0.07)
Family Mormoopidae				
Pteronotus davyi	2		Yucatan	< 0.07
Pteronotus parnelli	2	3	Tropics	< 0.09 (0.07–0.11)
Pteronotus suapurensis	1		Costa Rica	0.18
Mormoops megalophylla	1		Yucatan	< 0.08
Family Phyllostomatidae				
Subfamily Phyllostomatinae				
Micronycteris megalotis	1		Yucatan	< 0.09
Mimon cozumelae	1		Yucatan	< 0.09
Subfamily Glossophaginae				
Glossophaga soricina	9		Tropics	< 0.20 (0.07–0.30)
Glossophaga soricina		9	Costa Rica	< 0.18 (0.08–0.30)
Subfamily Carollinae				
Carollia brevicauda	3		Tropics	< 0.11 (0.08–0.14)
Subfamily Sturnirinae				
Sturnira lilium	1		Quintana Roo	< 0.07
Sturnira ludovici	1	1	Costa Rica	< (0.06–0.13)
Subfamily Stenoderminae				
Uroderma bilobatum	1	1	Costa Rica	< (0.08–0.14)
Chiroderma villosum	1		Quintana Roo	< 0.09
Artibeus jamaicensis	4	13	Tropics	< 0.07 (0.03–0.14)
Artibeus lituratus	2	3	Costa Rica	< 0.07 (0.05–0.09)
Artibeus phaeotis (infant)	1		Quintana Roo	< 0.15
Artibeus toltecus	1	3	Costa Rica	< 0.10 (0.06–0.14)
Subfamily Desmodontinae				
Desmodus rotundus		1	Yucatan	< 0.07
Diphylla ecaudata		1	Yucatan	< 0.09
Family Natalidae				
Natalus stramineus	1		Yucatan	< 0.20
Family Vespertilionidae				
Myotis keaysi	1		Yucatan	< 0.20
Myotis leibii		3	South Dakota	Nil‡
Myotis leibii (infants)	3	2	South Dakota	Nil‡
Myotis lucifugus	3	16	Minnesota	Nil‡
Myotis lucifugus	1		Minnesota	< 0.18
Myotis velifer	1		Kansas	< 0.30
Myotis riparius		1	Costa Rica	0.51
Eptesicus furinalis	1		Yucatan	< 0.14
Eptesicus fuscus	8	6 (+3 unknown)	Minnesota	< 0.07 (0.05–0.25)
Lasiurus ega		2	Yucatan	< (0.08–0.14)
Lasiurus intermedius		1	Quintana Roo	< 0.18
Plecotus townsendii	1		Montana	< 0.30
Family Molossidae				
Molossus ater		1	Yucatan	< 0.08
Molossus sinaloae	1		Yucatan	< 0.07
Promops centralis	1		Yucatan	< 0.09
Eumops glaucinus	1		Yucatan	< 0.09

* Bats were obtained from natural habitats in Yucatan and Quintana Roo, Mexico in April and May, Costa Rica in August and September and various places within the USA primarily during summer. Locality of collection sites is specified to state or country, except where our values represent specimens from two or more tropical localities, in which case locality is designated tropics. Livers were removed immediately after killing, frozen quickly and shipped and held in the frozen state until assayed. Voucher specimens of all species are preserved in the Bell Museum of Natural History.

† Analysed by method of Ayaz *et al.*[6] except as noted, and expressed as μmol ascorbate per g liver per h. Individual whole livers weighing 0.06–1.00 g were homogenised in 5–10 ml of buffer containing 0.2 % sodium deoxycholate. The homogenate was centrifuged at 20,000*g* for 30 min and sample of the supernatant was incubated for 30 min with L-gulonolactone (5.6 mM). We encountered no interference attributable to lactonases present in the homogenate[5], and addition of sodium pyrophosphate[5] did not affect the apparent activity of the enzyme. Ascorbate produced was estimated by the Roe–Kuether colorimetric method. Blanks were made by omitting L-gulonolactone from the incubation mixture. The net absorbance (sample minus blank) was above the value of 0.01, which we consider the minimum absorbance detectable with precision, for only three specimens (0.011, 0.025 and 0.011 for *Pteronotus suapurensis*, *Myotis riparius* and one *Artibeus jamaicensis*, respectively). Calculated values of L-gulonolactone oxidase activity are presented for these three; for all others the net absorbance was less than 0.01 and activities are reported (individual values or means and ranges) as falling below the values calculated for the respective liver weights if the net absorbance has been 0.01.

‡ Analysed by microsomal technique of Chatterjee[5].

of some bat livers. Consequently, we cannot deny unequivocally the presence of enzyme at levels up to the values given in Table 1. These values, however, are similar to those calculated in like manner for guinea pigs[6], a species classically considered to lack the ability to synthesise ascorbate[7].

We do not think that the apparent lack of enzyme activity shown in Table 1 resulted from loss in storage of the livers before analysis because some livers were analysed immediately after removal and others were transported and stored with rodent livers, which invariably exhibited activity levels normal for the species. No relationship was observed within the Chiroptera for change in biosynthetic ability with age (infant *Myotis leibii* and *Artibeus phaeotis* did not have L-gulonolactone oxidase) or sex (note especially *Glossophaga soricina* in Table 1).

Giroud *et al.*[8] demonstrated the presence of ascorbate in adrenals, kidney, and liver of bats of the genera *Plecotus, Rhinolophus* and *Eptesicus* We calculated mean ascorbate levels in mg per g tissue for five pooled samples of each tissue from ten young-of-the-year and two adult *Eptesicus fuscus* caught in late August as follows: adrenal, 0.29; spleen, 0.31; kidney, 0.10; liver, 0.20; testes (seven males), 0 19. Blood of the same bats contained 0.6–0.9 mg ascorbate per 100 ml. These values are comparable with those of mammals known to be synthesisers[8], and together with those for *Myotis lucifugus*[9] they demonstrate that bats are neither deficient in ascorbate nor adapted to survival in the absence of that molecule.

We investigated the hypotheses (1) that bats synthesise ascorbate in some tissue other than liver or (2) that they do so in liver *in vivo* but the reaction is somehow inhibited *in vitro*. Pooled samples of kidney tissue of *Myotis lucifugus* and *Eptesicus fuscus* were examined for L-gulonolactone oxidase using the microsomal[5] and deoxycholate[6] techniques, respectively. No enzyme activity was detected for either species. To test for an inhibitor, an homogenate of liver of *Eptesicus fuscus* was mixed 5 : 1 with one of rat. The enzyme activity of the rat liver was 12.4 μmol per g liver per h when assayed alone and 12.9 μmol per g liver per h when assayed in the mixture with bat liver. The possibility that bats synthesise ascorbate by a pathway that does not involve L-gulonolactone oxidase was not investigated.

The lack of L-gulonolactone oxidase in bat liver is strong presumptive evidence of lack of ability to synthesise L-ascorbic acid Evidence of deficiency on feeding ascorbate-free diets would provide confirmation, but all previously confirmed cases of lack of synthetic ability in vertebrates are due to absence of L-gulonolactone oxidase. We conclude therefore that the bats examined do not synthesise ascorbic acid, and we anticipate that this will prove to be characteristic of all members of the Chiroptera.

In spite of some speculation[10,11], the selective pressures that have resulted in the loss of L-gulonolactone oxidase on at least three occasions during the evolution of the Mammalia are unknown, and not appropriate for discussion in this brief report. Regardless of the specific cause, the ability to synthesise an essential molecule should be lost only when an adequate supply of that molecule is available in the diet. It might then be predicted that species unable to synthesise would have limited potential to exploit new habitats and food sources and ultimately to radiate phylogenetically. Accordingly, the guinea pig is the only known non-synthesising rodent, and the anthropoid apes are relatively limited in number of species and generally are herbivorous or omnivorous Such is not the case for bats. Within our sample alone, species are represented whose primary foods are fish (*Noctilio*), fruit (*Artibeus* and others), pollen-nectar (*Glossophaga*), blood (the desmodontines) and insects (the mormoopids, natalids, vespertilionids and molossids)

The earliest bats were probably primarily insectivorous[12]. Although some chiroptologists would disagree and little fossil evidence exists, bats are most likely monophyletic—they have evolved from some common non-synthesising, insectivorous stock. It follows then that lack of the ability to synthesise L-ascorbate has not been an evolutionary handicap for many bat lineages. Bats may have selected as primary diets foods high in ascorbate, or they may be especially adapted to utilise ascorbate efficiently, or both. One obvious food resource known to be deficient in ascorbate is seeds[13], on which no bat relies for a primary dietary item.

Field work in Yucatan was supported by funds from the Society of the Sigma Xi and College of Biological Sciences of the University of Minnesota. K.M.A. was supported by a training grant from the National Institutes of Health. Robert M. Timm assisted us in the field and Peter Gonnella assisted with the assays.

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Received September 8, 1975, accepted February 23, 1976.

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Interaction of Walker 256 mammary carcinoma and foetal rat liver in organ culture inhibits enzyme maturation

WHEN tumour tissue is implanted subcutaneously into an animal the enzymatic composition of the host liver changes so as to resemble that of well differentiated hepatomas[1-4]. When a tumour is implanted into a suckling rat (14–20 d old) the appearance of liver enzymes that occurs normally at that stage of development is severely inhibited[4] by as yet unknown mechanisms. We have investigated whether possible endocrine modification of the host by the implanted tumour is an obligatory step in the enzymatic modification of the host liver. We used cultured foetal liver, a system in which several enzymes have been reported to arise spontaneously[5-7]. Our results show that tumours can exert a direct effect on enzyme maturation of foetal liver in organ culture that is quantitatively comparable with the effect observed *in vivo*; they do not rule out a possible glandular involvement of the tumour-bearing host *in vivo*.

Livers from rat foetuses at 16–19 d gestation were removed aseptically, cut into 1–2 mm³ fragments, rinsed with Waymouth 751 medium and placed in groups of eight on top of 25 mm Metricel membrane filters, type GA-4 (0.8 μm), supported by sterile Gelfoam sponges (Upjohn) previously saturated with medium. Two such arrangements were maintained per 60-mm Falcon plastic culture dish. The solid transplantable mammary carcinoma Walker 256 was treated in the same way

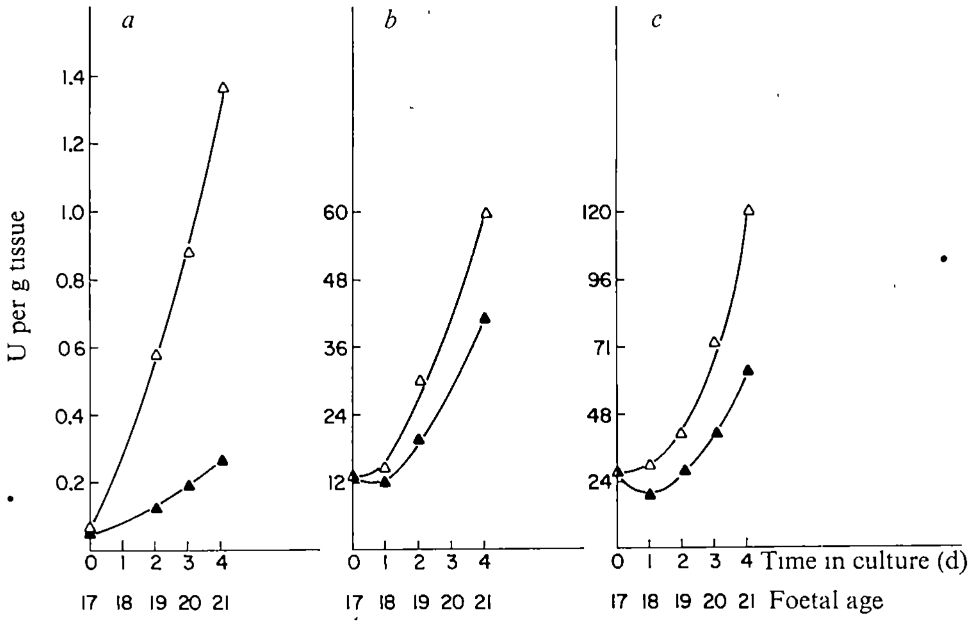

Fig. 1 Enzyme development in 17-d foetal liver explants. Falcon plastic dishes (60 mm) contained 4 ml Waymouth 751 medium supplemented with 10% heat-inactivated horse serum, 100 U of penicillin, 50 µg of streptomycin, 5 µg of fungizone per ml and, where indicated, with hydrocortisone (0.4 µg ml^{-1}). The *p*H of the medium was adjusted to 7.4 by addition of a 7.5% sodium bicarbonate solution and the dishes were incubated in Torbal anaerobic jars at 37 °C in an atmosphere of 70% oxygen, 5% CO_2, 25% nitrogen for a maximum of 5 d with daily changes of the medium. ▲, No additions to culture medium; △, hydrocortisone (0.4 µg per ml). *a*, TAT; *b*, AAT; *c*, arginase.

except that it was placed directly on to the sponges covered by the Metricel filters that supported the liver explants. Microscopy showed that the liver explants were not invaded by the tumour tissue and that the Metricel membranes prevented migration of cellular outgrowth. The viability of the tumour tissue was assessed by following the specific activity of thymidine kinase (EC 2.7.1.21) assayed by the method of Klemperer and Hayes[8], and that of the liver explants by following the accumulation of three enzymes: tryosine α-ketoglutarate transaminase (TAT) (EC 2.6.1.5) assayed by the method of Lin *et al.*[9], aspartate aminotransferase (AAT, EC 2.6.1.1) assayed according to Herzfeld and Greengard[10] and arginase (EC 3.5.3.1) determined according to Geyer and Dabich[11]. In addition, the maintenance of total weight (12–20 mg per filter) and total protein (3–5 mg per filter) for the duration of the culture proved to be a sensitive indicator of viability.

The development of AAT and arginase in the presence and absence of hydrocortisone in 17-d foetal liver explants is shown in Fig. 1. The kinetics of accumulation of these enzymes were similar in explants of 16-d-old foetuses but absolute values were about half of those in 17-d-old embryos. When livers of 19-d-old foetuses were explanted, initial values were higher but rose less sharply during culturing. The response of all three enzymes to hydrocortisone in the medium was the same at all ages. Although animals were time-mated, an 8–10 h uncertainty with respect to age (several litters were needed for each of the experiments reported) was sufficient to make standard deviations seem larger than those inherent in either the enzyme assays or the development of the enzymes in duplicate explants from the same litter. Representative data are therefore shown, and when tumour tissue was cocultured with foetal liver (below) experimental and control explants were from the same litter.

The behaviour of AAT in organ culture is similar to that *in vivo*: measurable levels appear on day 17 of gestation and a significant increase in activity has been reported in response to hydrocortisone[10]. The behaviour of TAT and arginase, on the other hand, differs from that *in vivo*: TAT does not appear until after birth, cannot be induced prematurely by hydrocortisone, and appears prematurely only when the young are delivered surgically before term[12]. Arginase activity can be detected from day 16 of gestation, but cortisol had no enhancing effect, during the prenatal period[13].

When foetal livers were cocultured with Walker 256 carcinoma (Fig. 2) the rate of accumulation of TAT decreased by 40%, and that of AAT by 25%. The development of arginase

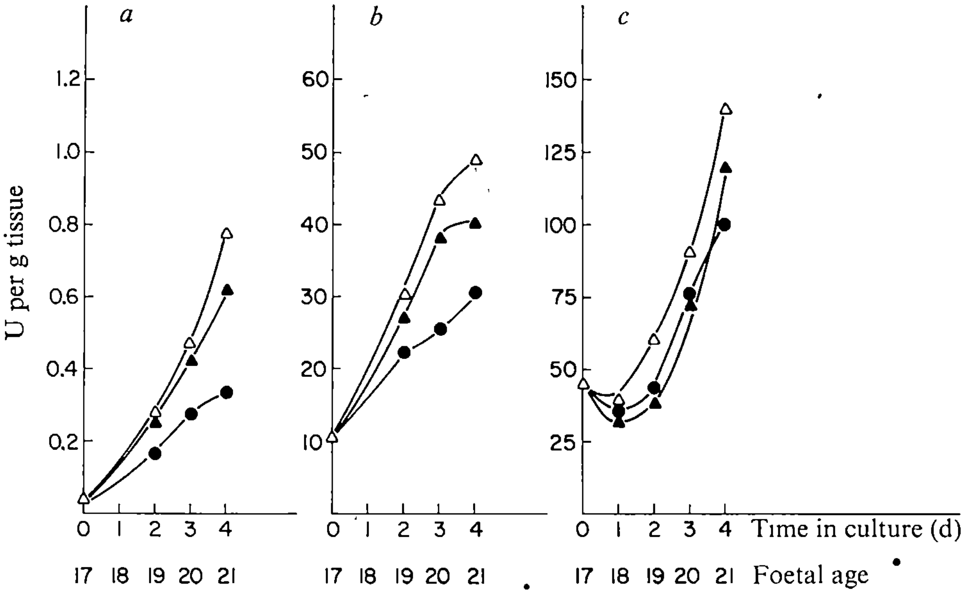

Fig. 2 Enzyme development in 17-d foetal liver explants cultured in the presence of Walker 256 mammary carcinoma. Livers were explanted as described for Fig. 1. All cultures were supplemented with 2 mM glutamine and hydrocortisone (0.4 µg ml^{-1}). ▲, Foetal liver; ●, foetal liver in the presence of tumour tissue; △, foetal liver in the presence of mammary tissue. *a*, TAT; *b*, AAT; *c*, arginase.

was not affected, precluding the interpretation that the tumour exhausted an essential component of the medium. This possibility is further made unlikely because liver explants cultured in the presence of tumour tissue consistently increased in total mass and protein by 15–20%, whereas controls did not.

All culture dishes containing Walker carcinoma (and those serving as controls) were supplemented with additional 2 mM glutamine (in view of the high glutaminase content of tumours) and with hydrocortisone (0.4 µg ml^{-1}). The inhibitory effect of the tumour on the rate of enzyme accumulation in the liver explants did not depend on the presence of the corticoid in the medium; rather, in initial mixed culture experiments without hydrocortisone, enzyme levels in the cocultured liver explants were too low to be measured accurately.

Table 1 Enzyme development in 19-d foetal liver explants

Days in culture	Foetal age	Additions to culture medium: None, Injected	Additions to culture medium: None, Controls	Cortisol (0.4 µg ml^{-1}), Injected	Cortisol (0.4 µg ml^{-1}), Controls
			TAT		
0	19	0.03	0.03	0.03	0.03
1	20	—	—	—	—
2	21	0.31	0.14	0.74	0.28
3	22	0.54	0.23	1.06	0.67
4	23	0.44	0.39	1.25	0.95
			AAT		
0	19	23.7	18.3	23.7	18.3
1	20	—	—	—	—
2	21	48.2	30.0	64.3	42.0
3	22	66.5	41.8	78.1	62.7
4	23	60.5	56.6	92.6	76.0
			Arginase		
0	19	26	21	26	21
1	20	37	13	62	47
2	21	90	32	142	90
3	22	83	59	217	158
4	23	70	71	248	174

Foetuses in one uterine horn were injected with 25 µg cortisol each, 24 h before their livers were explanted as described in the legend to Fig. 1; non-injected foetuses from the other uterine horn served as controls. Activities are given in units per g tissue.

To ascertain whether the inhibition of TAT and AAT accumulation was tumour specific, foetal livers were cultured in conditions identical to those described, but with mammary tissue from a pregnant dam instead of tumour. The activities of all three enzymes were always 10–20% higher than in controls (foetal liver explants alone). We cannot explain this observation; however, a differentiating effect of mammary mesenchyme on tumour tissue explants has been reported[14].

The inhibitory effect of the tumour on the enzymatic maturation of the liver explants was reversible and depended on the continued survival of the tumour in culture. When the sponges supporting the tumour tissue were replaced by empty sponges on day 3 of culture, enzyme accumulation resumed at the rate of controls. Similarly in some experiments in which the tumour did not survive as long as the liver explants (as ascertained by thymidine kinase activity measurements), accumulation of the indicator enzymes resumed at the rate of controls.

Because the cultures containing the tumour had to be maintained in the presence of hydrocortisone to obtain detectable enzyme levels in the cocultured livers, and all three enzymes responded to the hormone with an increased rate of accumulation in organ culture although two of them did not respond in this way *in utero*, it seemed that the corticoid may serve some nonspecific function in the system, perhaps the maintenance of tissue viability, similar to that of certain serum factors[15]. To test this possibility we injected each of several foetuses *in utero* with 25 µg hydrocortisone 24 h before their livers were explanted into media with and without hydrocortisone. The results of a representative experiment (Table 1) show that enzyme levels in cultures from 19-d-old foetuses which had received hydrocortisone *in utero* were similar for 2–3 d in culture to those which were explanted from uninjected controls into medium containing hydrocortisone. The rate of enzyme accumulation in the injected samples then slowed, whereas explants maintained on hydrocortisone with daily medium changes continued to respond with the same rate of enzyme accumulation; those injected and maintained on hydrocortisone showed the greatest response, particularly arginase. When foetuses were injected with hydrocortisone on day 16 of gestation and their livers were explanted 24 h later into media with and without hydrocortisone, the injected hormone was ineffective (data not shown). This argues against the possibility that the injected hormone had some lasting, nonspecific effect; rather it suggests that hydrocortisone has a specific inducing effect if administered when the liver is competent to initiate *in utero* the processes which culminate in enzyme production *in vitro*.

Our findings suggest that the Walker 256 carcinoma produces a diffusable factor (or factors) with an inhibitory effect on the rate of accumulation of some, but not all, enzymes that develop in embryonic liver maintained in organ culture. The effect is specific for Walker carcinoma tissue and its maintenance depends on the continued survival of the tumour in culture. The apparent rapid inactivation of the factor(s) in this system need not signify an inherent instability precluding its isolation; serum factors that withstand remarkably harsh conditions during their purification are known to be inactivated rapidly by cells in culture[16]. While the endocrine state of a tumour-bearing animal is undoubtedly modified, our results show that Walker carcinoma tissue exerts a readily measurable direct effect on developing liver in organ culture, that is without the intervention of endocrine changes. The system described is therefore well suited as an assay for the isolation of the pertinent factor(s) and for the study of its mechanism of action.

This work was supported by a grant from the US Public Health Services.

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Mechanism for suppression of cellular biosynthesis of prostaglandins

BIOSYNTHESIS of prostaglandins (PGs) from unsaturated fatty acid precursors involves a complex sequence of reactions that seem to proceed rapidly in response to physiological stimuli. Studies *in vitro* have revealed a capacity for biosynthesis greater than would be expected from measures of tissue content[1,2] or daily prostaglandin (PG) production[3]. Biosynthesis requires release of esterified precursor from tissue lipids[4,5], and control of the hydrolytic event may be a major means of controlling PG biosynthesis (for example, in brain[6] or spleen[7]). Another possible type of regulation is control of cyclo-oxygenase activity. Many chemical agents have been examined as modifiers of the PG-forming oxygenase (for example, reviews in refs 8 and 9). The enzyme can be inhibited by fatty acids[10] although they appear only in limited amounts in the cytosol. In addition, inhibition of the oxygenation reaction has been observed *in vitro* with added glutathione peroxidase (GSP) and reduced glutathione (GSH)[11–14]. We have further investigated this form of enzymatic regulation, and propose that it inhibits by destroying an essential activator of the oxygenase which forms the PGs and thromboxanes.

Supernatant (BSN) obtained from centrifugation of homogenates of bovine vesicular glands at 105,000*g* for 90 min gave 60% inhibition of oxygenation of arachidonic acid by bovine vesicular gland microsomes, and 40% inhibition of the activity of an acetone powder preparation from sheep vesicular glands. Since the bovine oxygenase showed only 12% of the specific activity of that from sheep, and the supernatant from sheep vesicular glands gave little inhibition of oxygenase preparations, the most active inhibitory and oxygenating preparations were used in subsequent experiments (Table 1). When sodium cyanide, an inhibitor of PG synthesis[15,16], was in the oxygenase assay mixture at a concentration (0.5 mM) that gave about 45% inhibition of oxygenation in the absence of added BSN, a synergistic inhibition was observed that was proportional to the amount of BSN when < 0.1 ml of BSN was present, and was complete with ≥ 0.1 ml of BSN. The BSN was only about half as inhibitory when additional GSH was omitted from the assay, and did not inhibit the oxygenase when 1.0 mM *N*-ethylmaleimide (NEM, which combined with the necessary mercaptan group of GSH) was added. Oxygenase activity could also be recovered when NEM was added to a reaction mixture several minutes after total inhibition by 0.1 ml of BSN. The total extent of reaction before and after addition of NEM was the same for all concentrations of BSN used, indicating that the capacity of the oxygenase for production of PGs and related derivatives was not irreversibly impaired by BSN.

When radioactive eicosatetraenoic acid (150 nmol) was incubated with the oxygenase preparation, 69–74% of the product formed migrated with ^{3}H-PGF$_{2\alpha}$ (R_f = 0.05–0.20) and ^{3}H-PGE$_2$ (R_f = 0.20–0.45) standards, 12–17% with the region associated with PGD$_2$, PGG$_2$ and PGH$_2$ (R_f = 0.30–0.45) and 10–19% with the hydroxy acid standard (R_f = 0.45–0.60). The relative amounts of product formed were similar in the presence of GSH and the BSN with or without NEM present. In the presence of 4 mM $SnCl_2$, 73–80% of the product migrated with PGF$_{2\alpha}$ (R_f = 0.05–0.20) and 6–19% with PGE$_2$ (R_f = 0.20–0.29) standards.

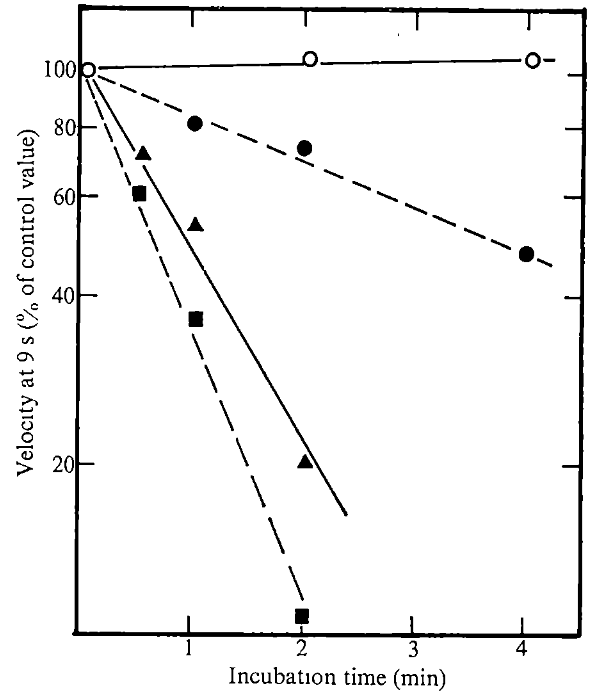

Fig. 1 Time-dependent destruction of the oxygenase activator by BSN and GSP. Activator was generated from 65 μM arachidonic acid with 350 μg of sheep vesicular gland dioxygenase in 3 ml of 0.1 M Tris chloride at *p*H 8.5, containing 0.67 mM phenol and NaCN, as indicated below. After 4 min of reaction and cessation of oxygen consumption, 500 μM GSH and the BSN or GSP solution were added. Then, after various times, the oxygenase reaction was initiated by addition of 350 μg of vesicular gland enzyme, and initial velocities were measured using an oxygen electrode equipped with an electronic differentiator. The value for the oxygenase velocity at 9 s is a measure of the amount of activator[16,18]. ○, 0.5 mM NaCN+heat-treated GSP; ●, 0.5 mM NaCN+120 U of GSP; ■, 1.0 mM NaCN+150 U of GSP, ▲, 0.5 mM NaCN+0.015 ml of BSN.

We have proposed[14,16] that the oxygenase required a hydroperoxide activator and PG production may be inhibited by removal of activator molecules by GSP. Figure 1 shows that very low levels of either BSN or GSP destroy the activator in a few minutes.

The inhibitory agent in the cytosol of bovine glands (BSN) had several properties similar to those of the GSP purified from rat liver[17] or bovine blood[18]. Both GSP and BSN were dependent on added GSH for maximal inhibitory ability (and added NEM,

Table 1 Inhibition of sheep vesicular gland dioxygenase by BSN

Initial reaction conditions	After addition of dioxygenase		After addition of 1 mM NEM	
	Optimal velocity (μmol O$_2$ min^{-1} mg^{-1})	Extent (μmol O$_2$)	Optimal velocity (μmol O$_2$ min^{-1} mg^{-1})	Extent (μmol O$_2$)
Control	470	46	0	0
+50 μl BSN	430	41	50	7
+100 μl BSN	270	28	145	16
+0.5 mM NaCN	289	45	0	0
+0.5 mM NaCN+50 μl BSN	182	29	74	16
+0.5 mM NaCN+70 μl BSN	89	19	161	22
+0.5 mM NaCN+100 μl BSN	0	0	239	38
+0.5 mM NaCN+100 μl heat-treated BSN	222	32	124	12
+0.5 mM NaCN+100 μl BSN+1 mM NEM	318	47	0	0
+0.5 mM NaCN+100 μl BSN+no exogenous GSH	205	43	0	0
+0.5 mM NaCN at *p*H 7.0	420	42	0	0
+0.5 mM NaCN+70 μl BSN at *p*H = 7.0	277	29	17	22

BSN was obtained by homogenising 100 g of bovine seminal vesicles in 200 ml of 0.1 M Tris-HCl at *p*H 8.5. The homogenate was centrifuged at 10,000*g* for 15 min and the supernatant was filtered through cheesecloth. This supernatant was centrifuged for 90 min at 105,000*g* and the resultant supernatant was removed and stored at −20 °C for later assay. Assays were made in 3.0 ml of 0.1 M Tris-HCl at *p*H 8.5 (except where otherwise indicated the *p*H 7.0 buffer was 0.1 M EDTA) containing 0.67 mM phenol, 0.5 mM reduced glutathione, 65 μM arachidonic acid and other additions as indicated. Reaction was initiated by addition of 375 μg of phenol-activated sheep vesicular gland enzyme. After 3–5 min, 1.0 mM *N*-ethylmalemide was added to the reaction mixtures. Dioxygenase activity was monitored with an oxygen electrode equipped with an electronic differentiator.

which removes GSH, eliminated inhibition by both); inhibitory in a concentration-dependent manner; synergistic with NaCN in the inhibition of PG formation; heat-labile and non-dialysable, destroying the activator of the oxygenase in a time-dependent manner (Fig. 1).

BSN also appeared most effective as an inhibitor at or near the *p*H optimum for GSP (*p*H > 8.5; see also refs 19–22). There was a slight difference in the amount of $(NH_4)_2SO_4$ required to salt the two inhibitors out of solution: approximately 68% of the total inhibitory ability of the BSN precipitated from solution between 0.33 and 0.50 saturation with $(NH_4)_2SO_4$ whereas about 60% of the measured GSP activity of rat liver supernatant was salted out between 0.45 and 0.65 $(NH_4)_2SO_4$. (Rat liver–GSP was obtained by extraction of protein precipitated in ethanol at −25 °C (ref. 17), whereas the BSN was a high speed supernatant and contained more protein per ml than the liver preparation which could explain the differences.) BSN, like GSP, catalysed the destruction of cumene hydroperoxide in the presence of reduced GSH (8 U peroxidase activity per µl of BSN—see ref. 18 for assay conditions and definition of units).

The apparent K_m for GSH may vary greatly depending on the initial steady-state concentration of the peroxide substrate[22,23], suggesting that as the local physiological concentrations of hydroperoxide substrate approach 1µM the *in vivo* level of GSH (usually between 100 µM and 5 mM (refs 24 and 25) may be close to its K_m for GSP. Thus, metabolic alterations of the intracellular concentrations of GSH within this concentration range could alter directly the effective peroxidase activity and thereby act as an intracellular modulator of the activity of the fatty acid oxygenase(s) that form the PGs and thromboxanes. Homogenisation of a tissue *in vitro* also gives dilution of the cytosolic GSP and GSH to concentrations that are less inhibitory than those *in vivo*.

Formation of PGs *in vitro* has been reported to be inhibited in a dose-dependent manner by a component of the supernatant obtained during the preparation of microsomes from bovine seminal vesicles[26,27] and rabbit renal medulla[28], but the nature of the inhibition was unclear. Inhibition by a soluble component from tissue preparations may also be inferred from the data from other studies with these and other tissues[29–32], that is, total product found in one or more of the PG fractions was significantly less when supernatant was included in the assays. Metabolic enzymes could lower accumulated levels of PG, particularly where the assay involves measurement of final concentrations of PG products (for example, ref. 32).

Although there is insufficient evidence to propose that GSP is the only inhibitor present in supernatant preparations, many assays for PG biosynthesis have included levels of GSH > 0.2 mM (refs 28–31), and a GSP-like activity could account for much of the inhibitor in supernatant reported in some of those *in vitro* studies. The observed effectiveness of the GSH-dependent peroxidase in blocking PG biosynthesis suggests that it has a significant physiological role in the many cell types in which it occurs. Thus, peroxidase activities could suppress PG biosynthesis by destruction of the essential activator as it is formed, so that (depending on the levels of peroxidase and essential cofactor, GSH) some dioxygenase molecules are not activated and therefore cannot participate in PG production. A decreasing concentration of cellular GSH could be an important means for cells to modulate cyclo-oxygenase activity to give increased production of PGs and their related derivatives.

This research was supported in part by a fellowship (to H.W.C.) from the Medical Research Council of Canada and a grant from the US National Science Foundation.

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Received August 25, 1975, accepted February 17, 1976.

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Evidence for the proximity of sweet and bitter receptor sites

ALTHOUGH sweetness and bitterness are generally referred to as two of the common or basic tastes, it is by no means certain that they are, in fact, primary[1]. Furthermore, neurophysiological evidence has shown that response to several of the basic taste stimuli can occur in a single taste cell[2]. Much structural progress in the chemistry of sweetness has been made in the past decade through Shallenberger's hypothesis[3–5] that the sweetness of sugars depends on hydrogen bonds between the sugar molecule and receptor site. Our previous work[6–9] has indicated that sugar molecules and their simple derivatives are nearly always sweet, bitter or bitter–sweet. Bitter–sweet sugar molecules, like certain artificial sweeteners such as saccharin, have both bitterness and sweetness as intrinsic features of their molecules[10]—neither response can be altered by exhaustive stepwise purification of such compounds[10]. Bitter–sweet sugar molecules appear to be 'polarised' on taste receptors[9] so that one end of the molecule binds to sweet receptor sites and the other to bitter receptor sites. It is not clear, however, whether these molecules distribute themselves, some on sweet receptors

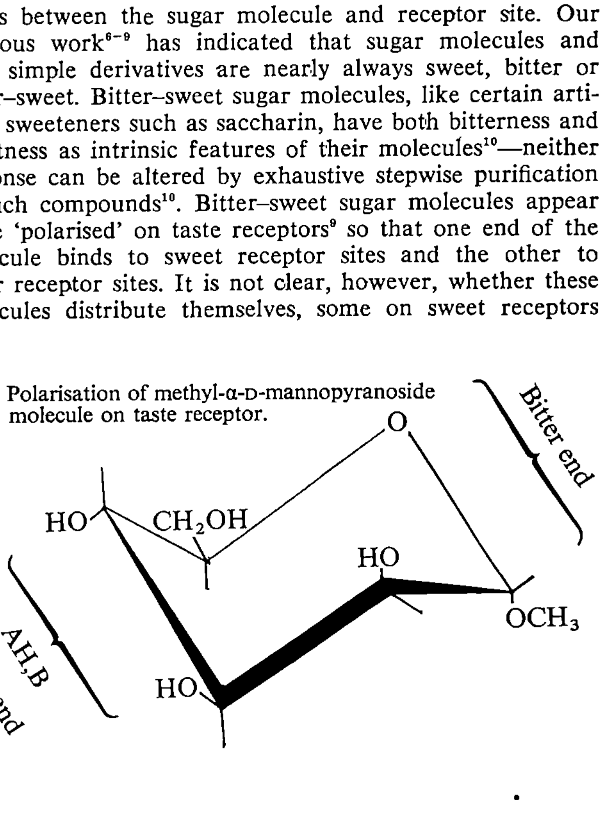

Fig. 1 Polarisation of methyl-α-D-mannopyranoside molecule on taste receptor.

Table 1 Perception of sweetness in sucrose (S) and methyl-α-D-mannopyranoside (M) before and after presaturation of taste receptors with 10 ml 0.0048% quinine sulphate solution*.

	Panellist	IT_{Sa} (7.5%)	IT_{Ma} (10%)	IT_{Sb} (10%)	IT_{Mb} (15%)	IT_{Sc} (12.5%)	IT_{Mc} (20%)		
Before presaturation	A	82	54	—	—	141	164		
	B	51	69	191	189	86	84		
	C	74	69	109	110	117	74		
	D	60	43	140	63	90	250		
	E	39	86	141	117	140	130		
	Mean	61	64	145	120	115	140	IT_{Sa} v IT_{Ma}	
	s.d.	17	16	34	52	26	71	IT_{Sb} v IT_{Mb}	NS
								IT_{Sc} v IT_{Mc}	
After presaturation	A	53	13	80	32	140	24		
	B	108	44	83	71	109	85		
	C	190	74	104	87	195	76		
	D	163	76	165	60	173	64		
	E	132	55	55	33	90	34		
	Mean	129	52	97	57	141	57	IT_{Sa} v IT_{Ma} $P<0.002$	
	s.d.	41	15	19	24	43	27	IT_{Sb} v IT_{Mb} $0.01<P<0.05$	
								IT_{Sc} v IT_{Ma} $0.002<P<0.01$	

*Sweetness is listed as product of peak subjective intensity (*I*, arbitrary scale 00 = zero sweetness to 5 = very sweet) and persistence (*T*) of response for sucrose (S) and methyl-α-D-mannopyranoside (M). Untrained panellists were simply selected on basis of recognising different concentrations employed in this experiment at one sitting Tap water was used for mouth rinsing and immediately afterwards panellists drank 1.0 ml of either sucrose or corresponding methyl-α-D-mannopyranoside solution, recording their subjective intensities on a Telsac Type X moving chart recorder travelling at 12.0 min^{-1} as soon as the solution had been imbibed. Only sweetness was recorded, bitterness being ignored. For tasting after presaturation of bitter receptor sites, panelists kept the 10 ml 0.0048% quinine sulphate solution in mouth for 10 s, then spat out, and immediately drank 1.0 ml of either sucrose or corresponding methyl-α-D-mannopyranoside solution, recording subjective intensity and time as before. All recordings were continued until zero sweetness response was obtained The order of presentation of samples to panellists was randomised. An interval of 1 5 min was allowed between successive corresponding sample tasting, for the comparisons before presaturation, and 5 0 min for comparisons after presaturation. Significance was determined by Student's *t* test.

and some on bitter receptors, or whether a single molecule can span both sweet and bitter receptor sites simultaneously. We now report an experiment designed to distinguish between these possibilities, using a typical bitter–sweet and conformationally defined glycoside, methyl-α-D-mannopyranoside (Fig. 1.).

The subjective intensity of taste response in sweet[11] and bitter[12] molecules is known to be proportional to the logarithm of concentration at presaturation levels[11,12]. We have examined the persistence time (*T*) of the response as well as the peak subjective intensity (*I*) as functions of the concentration of stimulus presented to panellists. The product (*IT*) of these parameters obtained by a continuous trace on a moving chart recorder is a function of the area under the resulting 'taste' curve, and thus total gustatory response. In Table 1, the sweetness values of sucrose and methyl-α-D-mannopyranoside (measured as the product (*IT*) at three different concentrations, are listed before and after presaturation of the taste buds with quinine sulphate solution. At all three concentrations tested a significantly diminished sweetness response after presaturation with quinine sulphate solution is observed with methyl-α-D-mannopyranoside solution, but not with sucrose.

In Table 2 the bitterness values of quinine sulphate and methyl-α-D-mannopyranoside (again measured as the product *IT*) at two different concentrations are listed before and after presaturation of the taste buds with sucrose solution. At both concentrations a significantly diminished bitterness after presaturation with sucrose solution is observed with methyl-α-D-mannopyranoside but not with quinine sulphate. The results in both tables clearly indicate a lowered gustatory response to methyl-α-D-mannopyranoside by our panellists if their tongues are presaturated with either sweet or bitter substances. Indeed the effect at the lower concentration of bitterness investigated was so marked that four out of the five panellists could detect no bitterness at all after presaturation of their tongues with sucrose, whereas in the same conditions their response to a similarly bitter quinine sulphate solution was little affected.

It is possibly still an open question whether a taste re-

Table 2 Perception of bitterness in quinine sulphate (Q) and methyl α-D-mannopyranoside (M) before and after presaturation of taste receptors with 10 ml 50% sucrose solution*

	Panellist	IT_Q (0.0016%)	IT_M (3.5%)	IT_Q (0.0024%)	IT_M (4.5%)
Before presaturation	L	190	180	—	—
	M	48	104	48	260
	N	150	145	149	163
	O	23	178	72	186
	P	151	106	90	184
	Mean	112	142	90	198
	s.d.	73	37	43	43
		NS		$0.01 < P < 0.05$	
After presaturation	L	111	0	—	—
	M	58	14	57	56
	N	147	0	148	147
	O	0	0	138	9
	P	42	0	44	209
	Mean	72	3	97	105
	s.d.	58	6	54	90
		$P<0.002$		NS	

*Bitterness is listed as product of peak subjective intensity (*I*, arbitrary scale 0 = zero bitterness to 5 = very bitter) and persistence (*T*) of response for quinine sulphate (Q) and methyl-α-D-mannopyranoside (M). The method used was exactly as before, but for tasting after presaturation, panellists kept 10 ml 50% sucrose solution in mouth for 10 s before spitting out. An interval of at least 20 min was allowed between successive corresponding sample tasting, and significance was again determined by Student's *t* test.

sponse can be simply and mechanistically explained in terms of bonding forces between a stimulus molecule and a receptor, or whether a more complicated psychological explanation based on the decodifying of perceptual information[1] might give a truer description of the phenomenon. Many advances have been made[6–9,13–15] on the basis of the former hypothesis, working on the assumption that the sweetness of sugars arises from the existence of AH,B systems in their molecules which govern their ability to hydrogen bond to taste receptors. Sugars generally possess several possible α-glycol groupings meeting Shallenberger's[3–5] requirements for the AH,B system and with oxygen–oxygen interorbital distances of 2.86 Å, but evidence has accrued[6–9,13–15] to implicate the third and fourth hydroxyl groups of aldohexopyranosyl structures as having a significant and particular role in the sweetness response. By contrast that area of the molecule which surrounds the anomeric centre does not appear to influence sweetness[6,9,13] but does affect bitterness and these distinct gustatory features seem to be characteristic of a considerable number of different glycoside molecules[9,13].

Not all receptor sites are on the same receptor cell, and Ogawa *et al.*[16] have reported that 25% of fibres tested from the chorda tympani of rat and hamster responded to all four basic tests. Frank and Pfaffmann[17] have also shown in the glossopharyngeal nerves of rat that the majority of the fibres are not completely specific for any of the basic tastes, and only a small percentage responded to both sucrose and quinine. It is therefore clear that taste cells may vary in degree of specificity.

The results of this experiment can be interpreted on the basis of our hypothesis that sugar molecules are 'polarised' on taste receptors[8], to mean that the bitter/sweet glycoside attaches itself by a chelating action to the sweet and bitter receptor sites simultaneously. Impairment of either mode of attachment by presaturating the taste receptors with sweet or bitter molecules results in a lowered response. This also suggests that at least some of the sweet and bitter receptor sites might be extremely close to one another, probably within 3–4 Å.

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Received December 15, 1975; accepted February 26, 1976.

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Uptake and catabolism of low density lipoprotein by human lymphocytes

BROWN and Goldstein[1,2] have shown that when cultured human fibroblasts are incubated in the presence of low density lipoprotein (LDL), they take up some LDL, the apoprotein of LDL (apoLDL) is catabolised and cholesterol synthesis is inhibited. Experiments on the uptake of LDL as a function of LDL concentration have led to the conclusion[1,2] that normal fibroblasts have genetically controlled surface receptors with high affinity for apoLDL and that uptake of LDL by the receptors is essential for the regulation of cholesterol synthesis. These findings raise the possibility that receptor-mediated uptake by fibroblasts, and perhaps by other cells[3,4], has an important role in the regulation of apoLDL catabolism and of cholesterol synthesis *in vivo*. But the concentration of LDL protein required to saturate the LDL receptors on cultured fibroblasts (about 50 μg ml^{-1}) is less than half the probable concentration in interstitial fluid[5]. Moreover, whereas cultured fibroblasts from patients with familial hypercholesterolaemia (FH) in the homozygous form synthesise cholesterol at 50 times the normal rate, the rate of synthesis of cholesterol in the whole bodies of FH patients is usually normal[6] or subnormal[7]. For these reasons it seemed desirable to study the binding and catabolism of LDL by uncultured human cells. We used circulating lymphocytes because they can be obtained readily and repeatedly from healthy human subjects. Our results show that uncultured lymphocytes exhibit high capacity surface binding of LDL, but the binding curves show no evidence for the presence of high affinity receptors for LDL.

Lymphocytes, prepared from 300 ml of freshly drawn heparinised blood by density-gradient centrifugation[8], were washed four times in Eagle's MEM. LDL (density 1.020–1.050), prepared from normal human subjects[9], was labelled with ^{125}I by the iodine monochloride method[10]. Of the total ^{125}I in the labelled LDL, 5–7% was extractable with chloroform–methanol (2 : 1, v/v).

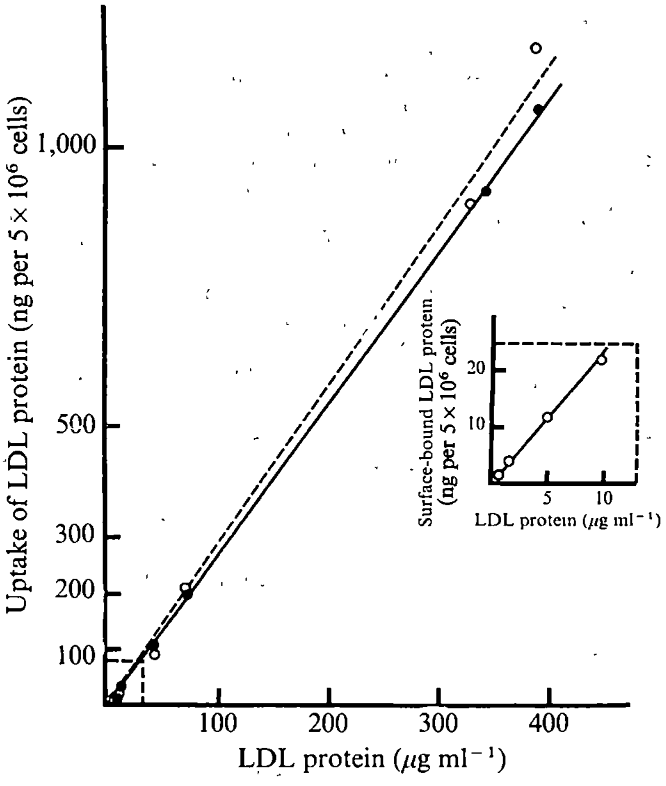

Fig. 1 Uptake of surface-bound (○) and internalised (●) LDL protein by human lymphocytes as a function of LDL concentration in the incubation medium. Inset shows expansion of curve for surface-bound LDL protein at low LDL concentrations. After incubation in duplicate with labelled LDL the cells were washed seven times before radioassay. Surface-bound and internalised LDL protein in the washed cells were estimated from the amounts of non-lipid ^{125}I releasable and non-releasable by trypsin respectively. The specific radioactivity of the LDL protein in the incubation medium was used to convert c.p.m. into ng of LDL protein taken up. Values shown are from one of five experiments with lymphocytes from five normal subjects. Experimental details are given in the text.

Binding of the labelled LDL by lymphocytes was investigated by incubating suspensions of cells 5×10^6 cells in 1 ml of MEM containing 1% bovine serum albumin (BSA) for 2 h at 25 °C in plastic tubes in the presence of ^{125}I-LDL. The cells were then separated from the medium by centrifugation. Radioactive breakdown products of the labelled apoLDL in the medium were estimated as trichloroacetic acid (TCA)-soluble radioactivity in the supernatant, after correction for radioiodide[11]. The cells were washed seven times with MEM to remove loosely adsorbed radioactive particles, transferred to fresh plastic tubes and assayed for radioactivity. Lipid-free radioactivity in the washed cells was fractionated into surface bound (^{125}I released by brief incubation with trypsin) and internalised (^{125}I not released by trypsin), essentially by the method of Bierman *et al.*[11].

Table 1 Effect of heparin on trypsin-releasable LDL protein bound to lymphocytes

Treatment	Incubation time (h)			
	1	3	4	5
No heparin	244	238	201	213
Heparin at 3.5 h			260	

All values are expressed in ng of LDL protein bound per 5×10^6 cells and are means of duplicate incubations. Lymphocytes were prelabelled by incubation with ^{125}I-LDL at 25 °C for 2 h in MEM containing 1% BSA. Loosely bound LDL was removed from the cells by repeated washing. Duplicate tubes containing the washed labelled cells were incubated for 1, 3, 4 and 5 h. Heparin (10 mg ml^{-1}) was added to one pair of tubes after 3.5 h and the tubes were incubated for a further 30 min. The amount of trypsin-releasable LDL protein bound to the cells at each interval was determined as described in the text. Binding of LDL by cultured fibroblasts has usually been studied at 4 or 37 °C. Our incubations were carried out at 25 °C because at this temperature lymphocytes catabolise apoLDL, while remaining viable for longer than at 37 °C.

When normal lymphocytes were incubated for 2 h with ^{125}I-LDL, uptake of trypsin-releasable LDL protein and of LDL protein not released by trypsin were both directly proportional to the concentration of LDL in the incubation medium over the range from 0.5 to 380 μg of LDL protein per ml (Fig. 1). No inflection in the curve relating uptake to LDL concentration could be discerned, even at the lowest concentrations (Fig. 1, inset). Thus the binding curves obtained in the conditions of these experiments gave no indication of the presence of high affinity LDL receptors with saturation at about 50 μg of LDL protein per ml. In all experiments, about half the total LDL protein taken up by the cells was internalised by the end of the incubation. There was some variation in the amount of LDL protein taken up at a given LDL concentration by lymphocytes from different normal subjects, but almost identical results were obtained when LDL from different subjects was tested against a single preparation of lymphocytes.

The accumulation of TCA-soluble radioactivity derived from the labelled LDL in the medium was determined after 2-h incubations with lymphocytes from normal subjects. In five preparations of lymphocytes the accumulation of radioactive breakdown products, expressed as a percentage of the total cell-associated radioactive protein (surface-bound plus internalised) recovered at the end of the incubation, averaged 4.8 ± 2.0 (s.d.) (range, 1.8–6.9).

The apparent lack of high affinity LDL receptors on lymphocytes could be due to irreversible saturation of binding sites by LDL before the cells are separated from the blood plasma. The binding curves for trypsin-releasable apoLDL, however, suggest that lymphocytes differ from cultured skin fibroblasts in being able to bind LDL to their surfaces by a mechanism that is not saturated at concentrations approaching 400 μg of apoLDL per ml. High capacity binding of LDL by lymphocytes was investigated further in the experiment shown in Table 1. Lymphocytes were incubated for 2 h at 25 °C in MEM containing 1% BSA and radioactive LDL (500 μg of apoLDL per ml). The cells were then washed seven times and incubated again for increasing periods in MEM containing 1% BSA but no radioactive LDL. Surface-bound LDL protein was assayed at each interval by the methods described above. There was no significant decrease in surface-bound LDL protein during the 5 h of incubation. When heparin (known to displace LDL from the LDL receptors on cultured fibroblasts[12]) was added to the cell suspension after incubation for 3.5 h, there was no displacement of surface-bound LDL protein during incubation for a further 30 min.

These results show that lymphocytes resemble cultured skin fibroblasts in their ability to take up LDL from an incubation medium and to degrade apoLDL. But lymphocytes seem to differ from cultured fibroblasts in being able to bind LDL to high capacity sites that are accessible to trypsin, but from which LDL cannot be displaced by heparin or by incubation in an LDL-free medium for 5 h. A surface-binding mechanism for LDL with high capacity seems more appropriate for circulating lymphocytes than one which is saturated at a concentration of 50 μg of apoLDL per ml, since the concentration of apoLDL in normal human plasma is at least 600 μg per ml.

We thank Mr P. Mistry and Mr D. N. Rudra for assistance.

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Received January 8; accepted February 18, 1976.

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Nuclear retention of receptor–oestrogen complex and nuclear acceptor sites

OESTROGEN-SENSITIVE cells contain cytoplasmic macromolecules called receptors[1,2]. They bind oestrogen to form complexes which are translocated to the nucleus where they may bind to specific acceptor sites[3–5]. This interaction of receptor–hormone complex with nuclear acceptor sites is assumed to be responsible for the stimulation of RNA, DNA and protein synthesis that results in growth of the tissue[3–5]. The demonstration of nuclear acceptor sites has been difficult and controversial[6,7]. We present here evidence that a limited number of receptor–oestrogen complexes are bound in the nucleus of rat uterine cells and that these sites may be specific nuclear acceptor sites.

Several investigators have shown that nuclear binding of receptor steroid complexes is a saturable phenomenon, thus suggesting that specific acceptor sites exist[7–14], while others claim that limited numbers of specific nuclear sites do not exist and that nuclear saturation is an artefact[6,15,16]. Much of this conflict probably stems from the difficulty inherent in the detection of a small number of specific binding sites in the presence of a large number of nonspecific sites[17].

This is especially true in view of the very "sticky" nature of the receptor–oestrogen complex after exposure to elevated temperatures (20–30 °C) at physiological salt concentrations[18]. The dissociation constant (K_d) for the binding of the receptor–oestrogen complex to various types of nuclei and non-biological surfaces, including glass, is 10^{-9} M at 25 °C (ref. 18). Thus, studies of the binding of the receptor–oestrogen complex to nuclei, chromatin and/or DNA in cell-free systems are susceptible to the error introduced by the masking effect of nonspecific binding.

Although the demonstration of nuclear acceptor binding is difficult *in vitro*, we have shown that a limited number of nuclear binding sites are involved in the production of maximal uterine growth[4,5,19]. We have also demonstrated that the long term retention of 1,000–3,000 receptor–oestrogen complexes per cell is a requirement of uterine growth[19,20]. Since uterine cells contain 15,000–20,000 receptor sites in the cytoplasm, it is apparent that only a fraction of these are required for maximal growth responses. These concepts and experiments are summarised in Fig. 1. We have suggested that the long term retention of the receptor–oestrogen complex in the nucleus is due to the binding of these complexes to a limited number of nuclear acceptor sites and that retention at these acceptor sites for more than 4–6 h is a requirement for true growth[5,21].

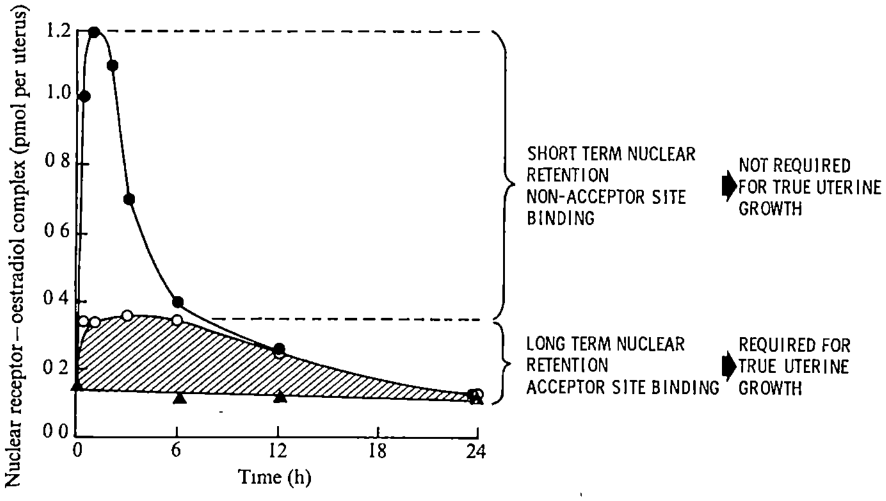

Fig. 1 Relationship between nuclear retention of the oestrogen receptor and uterine growth. Immature rats, 21–23 d old, were injected with either 0.1 or 2.5 μg of oestradiol and the accumulation and retention of the oestrogen receptor by the uterine nuclear fraction was examined by the ^{3}H-oestradiol exchange assay[4,26]. Uterine growth responses (DNA, RNA and protein content; wet and dry weight) were measured 24–48 h after injection (data not shown, see refs 4, 5, 19 and 20), and were stimulated maximally by 0.1 μg of oestradiol[4,5,19,20]. Therefore we conclude that 0.1–0.2 pmol of receptor per uterus is required for the stimulation of true uterine growth[20]. ●, 2.5 μg of oestradiol; ○, 0.1 μg of oestradiol; ▲, saline.

To test this hypothesis further we have used salt extraction of uterine nuclei to examine for differential extractability of the receptor–oestrogen complex. The rationale for the use of this technique was based on the observation, by several investigators, that extraction of nuclei with 0.3–0.4 M KCl does not remove all the nuclear-bound oestrogen[22–24], suggesting that some receptor–hormone complexes are bound more tightly than others. Immature rats 21–23 d old were injected with either a small dose (0.1 μg) or a large dose (2.5 μg) of oestradiol-17β. The animals were killed and their uteri removed 1 or 6 h later. A nuclear pellet was prepared by centrifugation of a uterine homogenate (30–50 mg ml^{-1}) at 800*g* for 10 min[25]. Although purified nuclei were not used, they behave in a fashion similar to that of this crude nuclear preparation (to be published elsewhere). The nuclear fraction was washed twice with cold TE buffer (0.01 M Tris, 0.001 M EDTA, *p*H 7.2, 4 °C). Salt extraction was performed by adding various concentrations of TK buffer (0.01 M Tris and 0.1–0.6 M KCl, *p*H 8.0, 4 °C) to the nuclear pellet with the mass to volume ratio maintained at 30–50 mg ml^{-1}. The nuclear pellets were resuspended in TK buffer and allowed to stand on ice, with mixing every 5–10 min, for 30 min. Nuclear fractions were centrifuged at 800*g* for 10 min and the KCl extract was decanted. TE buffer was added to the nuclear pellet (30–50 mg ml^{-1}) and the pellet was resuspended. If necessary, resuspension was accomplished by rehomogenisation of the pellet with a glass–Teflon homogeniser. The nuclear suspension was then added to small assay tubes (5–10 mg per tube) and the quantity of receptor in the nuclear pellet was measured by ^{3}H-oestradiol exchange[25].

The uterine nuclear fraction from rats that received 2.5 μg of oestradiol 1 h before they were killed contains a large number of receptor sites which are extractable with KCl concentrations less than 0.4 M (Fig. 2*a*). The quantity of receptor which remains in the nuclear fraction after exposure to KCl concentrations of 0.4 M or greater is approximately 0.1 pmol per uterus or 1,400 sites per cell. Thus, this small number of KCl-resistant receptor sites could represent those receptor sites which exhibit long term nuclear retention (Fig. 1). If this were true, the number of

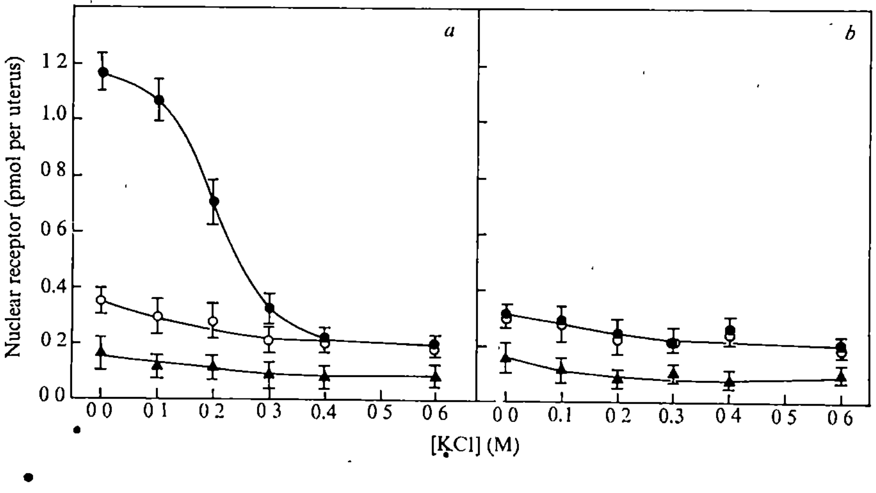

Fig. 2 Differential salt extraction of nuclear-bound oestrogen receptor. Immature rats were injected with oestradiol as described for Fig. 1. Uterine nuclear fractions were prepared 1 and 6 h after injection and differential KCl extraction was performed as described in the text. After extraction, the quantity of receptor which remained in the nuclear fraction was measured by the ^{3}H-oestradiol exchange assay[25]. *a*, 1 h; *b*, 6 h. Symbols as for Fig. 1.

KCl-resistant sites in animals receiving the large dose of hormone should equal the number receiving the small dose, since both doses produce the same number of long term retention sites (Fig. 1). As Fig. 2*a* shows, this seems to be the case. Likewise the number of KCl-resistant sites should be equal 6 h after injection of either dose of oestrogen and should be approximately the same as the number 1 h after the small dose. This also is the case, as Fig 2*b* shows. Therefore, we conclude that the number of receptor sites which remain in the nucleus after 0.4 M KCl extractions, are correlated with the number of sites which exhibit long term nuclear retention. The differential extraction of the receptor–oestrogen complex by KCl may reflect the binding of these complexes to different types of nuclear sites. Most (80–90%) of these nuclear receptor binding sites are of low affinity and extractable with KCl, whereas a minority (10–20%) are of high affinity and are non-extractable sites.

Other observations have suggested that receptor–hormone complexes manifest different characteristics once they have undergone nuclear binding. The rate of dissociation of the receptor–oestrogen complex is much slower when bound to chromatin than when free[26,27]. DeHertogh has observed a small pool of nuclear bound receptor *in vivo* which exhibits a relatively slow rate of oestradiol exchange when compared with the majority of receptor[23]. We have also observed two rates of exchange of the receptor–oestrogen complex when it is bound to the nucleus (unpublished). The exact relationship of these various observations to these reported here are not clear but they may reflect conformational changes in receptor–oestrogen complexes attendant on their association with specific nuclear acceptor sites. Such conformational alterations might result in associations of great affinity between receptor–hormone complexes and acceptor sites, thereby accounting for their resistance to KCl extraction. Since the number of these KCl-resistant nuclear sites is identical to that number of sites required for maximal uterine growth, we propose that these binding sites in the nucleus represent specific acceptor sites.

This work was supported by grants from the National Institutes of Health and the American Cancer Society. We than K. O'Conner and J. Kovar for technical assistance.

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Received November 24, 1975; accepted March 2, 1976.

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Interferon action may not be effected by induction of a cellular ribonuclease

Treatment of animal cells with interferon or polyinosinic–polycytidylic acid (pI : pC) can prevent multiplication of animal viruses. In the case of most of such viruses the interferon action consists of an inhibition of the viral translation. For some viruses, such as the DNA-containing Simian virus 40 (SV40), there is evidence for direct inhibition both of transcription[1–3] and translation[4,5]. Marcus *et al*[6] have shown, in chick embryo fibroblasts (CEF), that interferon or pI: pC are able to induce membrane-bound alkaline RNase activity. It was therefore proposed that degradation of viral RNA by membrane-associated RNase is indicative of a unifying concept of interferon action[6]. We attempted to determine whether the induction of interferon is paralleled by an induction of membrane-associated RNase activity in CEF and in primary African green monkey kidney (AGMK) cells. Our results show RNase induction in CEF but not in AGMK cells.

We have found a relatively high level of RNase associated with the membranes from unstimulated CEF compared with membranes from AGMK cells (see Table 1 and Fig. 1). Previous stimulation of CEF with pI : pC resulted in a sevenfold increase in RNase level. This level in the assay mixture (0.25 ml), containing 4 μg membrane protein, is equivalent to the activity of 30 ng ml^{-1} bovine RNase. These data obtained using CEF agree well with the results of Marcus *et al.*[6]. Stimulation of AGMK cells with pI:pC, however, failed to induce a significant increase in RNase level in those cells. Similar results with both CEF and AGMK cells were obtained when membranes were prepared according to the method of Joklik *et al.*[8].

The RNase induced in CEF also proved to be active using host-specific RNA as a substrate, indicating that the RNase does not show a specificity for viral RNA; this might be expected if it were involved in the action of interferon. We are, therefore, able to confirm the findings of Marcus *et al.*[6] for CEF, but we cannot support the

Table 1 Induction of interferon and isolation of membranes from CEF and AGMK cells

Interferon induction*		Membrane isolation	
Cell type and DEAE–dextran treatment (μg ml^{-1})	pI : pC treatment (μg ml^{-1})	Total membrane protein in 10^8 cells† (μg)	Membrane protein in 0.25 ml of RNase assay (μg)
AGMK	0	440	14.8
500	20	340	15.0
CEF	0	270	4.0
10	2	285	4.2

Membranes were prepared in discontinuous sucrose gradients[6,7]. Seven unequal membrane bands were visible. Band 3 (from top) consisting of a layer of smooth membranes, and band 4, containing rough and smooth membranes[7], were combined. These fractions were subsequently subjected to RNase assay. Substrates in tests[1] were ^{3}H-uridine-labelled AGMK cell RNA and *in vitro* transcribed ^{3}H-SV40 cRNA.

* Cells were treated with the mixture of DEAE–dextran and pI : pC for a 15-min period 18 h before membrane isolation[3]. This treatment renders more than 99% AGMK cells resistant to an infection with SV40 (multiplicity of infection = 10) and renders CEF resistant to infection with vesicular stomatitis virus (multiplicity of infection = 1) for a period of 24–48 h.

† Gradient fractions 3 and 4 (ref. 7) were combined.

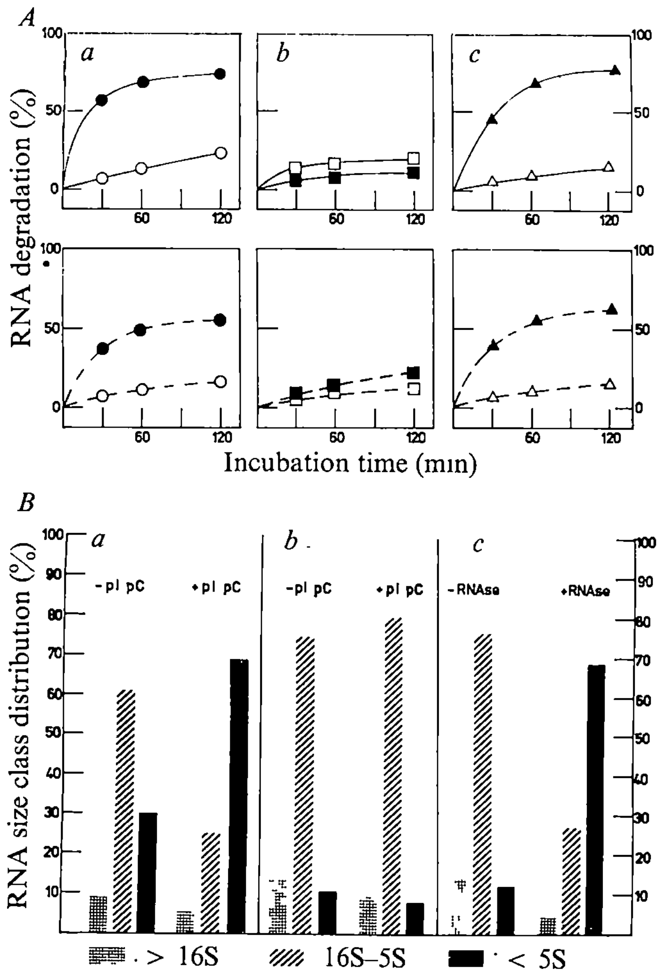

Fig. 1 *A*, Degradation of ^{3}H-RNA after incubation with membrane fractions from *a*, CEF (○, ●) and *b*, AGMK cells (□, ■) pretreated with pI : pC (closed symbols) and untreated (open symbols). Assay mixture contained (total volume 0.25 ml) 0.1 ml membrane protein preparation (Table 1), 0.1 M Tris-HCl (*p*H 8.2), 7.5 mM Mg^{2+}, 1 mM EDTA, and 2.5×10^3 c.p.m. ^{3}H-RNA substrate. Incubation temperature was 37 °C. Substrate was ^{3}H-labelled SV40 cRNA[9] (specific activity 1×10^4 c.p.m. μg^{-1}, ———); and ^{3}H-RNA from ^{3}H-uridine-labelled AGMK cell RNA (specific activity 1.5×10^4 c.p.m. μg^{-1}, – – – – –). Degradation of ^{3}H-RNA during incubation was assayed by scintillation counting of TCA precipitates on Whatman GF/C filters. *c*, Controls: incubation with 30 ng ml^{-1} bovine RNase (EC 3.1.4.22; ▲) and without RNAse addition (△). *B*, Distribution of three ^{3}H-SV40 cRNA size classes after 120-min incubation period with membrane protein (Table 1) from pI : pC-treated (+pI : pC) and untreated (−pI : pC) CEF (*a*) and AGMK cells (*b*) *c*, Controls: incubation with bovine RNase as above. Size classes of ^{3}H-SV40 cRNA were determined by centrifugation in formaldehyde–sucrose gradients[9].

generalisation drawn from this phenomenon that resulted in the unifying concept of interferon action.

An inhibition by interferon of SV40 tumour antigen formation in monkey cells microinjected with *in vitro* transscribed SV40-specific RNA has been found[4,5]. In terms of this inhibition, the failure of pI : pC to induce in AGMK cells a membrane-associated RNase activity, suggests that inhibition of translation is unlikely to be affected by induction of membrane-associated RNase. It has also been found that interferon treatment is also ineffective in inducing RNase activity in embryonic hamster cells. P. I. Marcus (personal communication) has obtained the same results with cells from quails and ducks.

We thank Miss Erika Koch for assistance and Dr H. Werchau for advice in preparing membranes. Supported by the Deutsche Forschungsgemeinschaft.

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Received November 16, 1975; accepted March 1, 1976.

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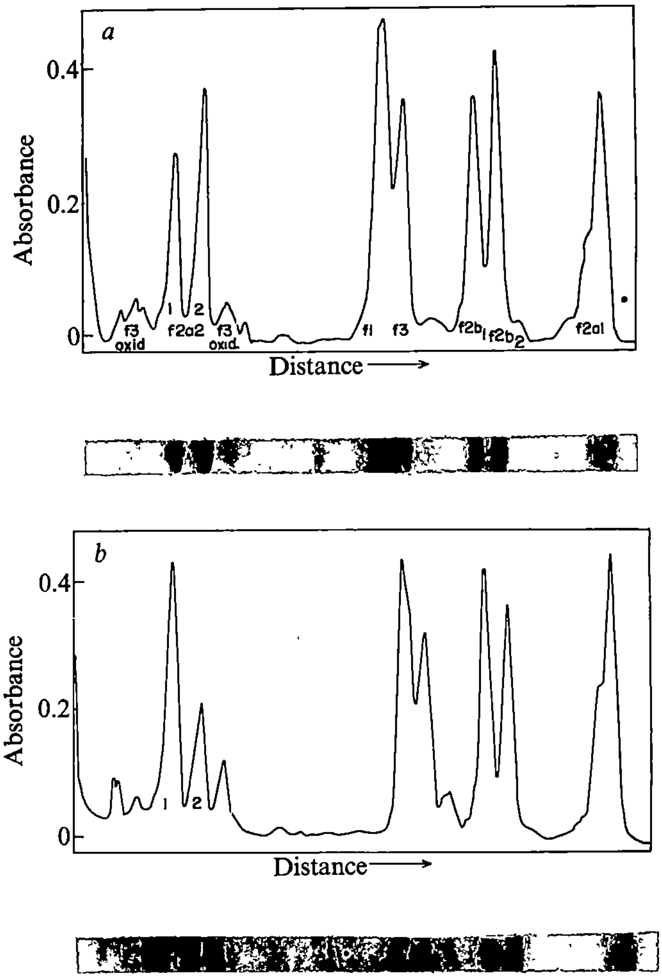
Changes in histone f2a2 associated with proliferation of Friend leukaemic cells

HISTONE–HISTONE interactions with DNA form the basic structural unit of chromatin[1–3], and quantitative changes in the cellular histones correlate with alterations in chromatin structure[4,5]. More subtle changes in chromatin structure follow chemical modification of the histones. These changes may enable genes to assume proper configuration for specific regulation by the non-histone proteins.

Growth of Friend cells *in vitro* with 1–2% dimethylsulphoxide (DMSO) induces globin mRNA synthesis[6,7], the appearance of erythrocyte membrane antigen[8], an increase in haem synthesis and iron uptake[8], an alteration in purine metabolism[9] and morphological changes characteristic of erythroid differentiation[10]. For this response the cells must complete S phase in the presence of 1–2% internal concentration of DMSO[11]. Evidence suggests that DMSO or a metabolic product acts at the level of the chromatin, causing biochemical and physical alteration in DNA–protein interactions[12–14]. If this is true, perhaps the ability of the cell to respond to DMSO depends on a particular chromatin structure. We have established DMSO-inducible and DMSO-non-inducible cell lines *in vitro* from subcutaneous tumours derived from a Friend leukaemic spleen in DBA/2J mice. Analysis of the histones from these cell lines has demonstrated a striking difference in histone f2a2 during establishment of the lines in culture, especially when DMSO-responsive and unresponsive cells are compared.

In acid–urea gels[15], no difference in the kinds or amounts of histones from the different cell types was found. Separation on acid–urea Triton gels[16,17], however, resulted in a finer resolution of the histones, and demonstrated a marked difference in the f2a2 histone subfractions from different Friend leukaemic cells (Table 1). In these gels, histone f2a2 from normal spleen and liver tissue separated into two fractions, $f2a2_1$ and $f2a2_2$, in a ratio of 3 : 1 (Table 1). A similar f2a2 ratio was found for leukaemic spleen tissue or subcutaneous tumour cells taken from the mouse. Friend tumour cells established as *in vitro* cell lines (for example, TP46, TP63), however, showed an increase in $f2a2_2$ and a $f2a2_1 : f2a2_2$ ratio of about 2 : 1. These freshly established cell lines were unresponsive to DMSO (Table 1). After several months' passage in culture, these cell lines began to show a response to DMSO with synthesis of haemoglobin. Coincident with this change in response to DMSO was a further increase in $f2a2_2$ with the ratio of $f2a2_1 : f2a2_2$ now approaching 1 : 1 (Fig. 1, Table 1). Less extensive differences were observed in f2b and f3 histones. The f2a2 ratio was not altered when DMSO-responsive or unresponsive cells were grown subcutaneously into a tumour in a mouse (C17, Table 1). Furthermore, in contrast to the gradual adaptation of Friend tumour cells to tissue culture, these tumour cells derived from tissue culture, with increased $f2a2_2$, adapted immediately to growth *in vitro* and retained their original response to DMSO.

Fig. 1 Histones from C17 cloned cell line (*a*), and leukaemic spleens (*b*). Tissue culture cell lines were obtained from tumours initiated by placing a piece of Friend leukaemic spleen subcutaneously in a mouse. Cells were grown in basal media, Earle's salts (Gibco) containing penicillin and streptomycin and 15% foetal calf serum. For isolation of nuclei and histone extraction, we used the following procedure. Cells collected by centrifugation were resuspended at 10^8 cells per ml in buffer 1 (0.05 M Tris-HCl, *p*H 7.5, 5 mM $MgCl_2$, 0.02 M KCl, 0.25 M sucrose, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 5 mM sodium bisulphite) and incubated at 4 °C for 5 min before being sedimented by centrifugation (5 min at 2,500 r.p.m. in a SS34 rotor (Sorvall)). This procedure was repeated. Cells were then suspended in 10 volumes of buffer 2 (5 mM Tris, *p*H 7.1, 5 mM $MgCl_2$, 0.05 mM dithiothreitol, 0.1 mM PMSF) and Triton X-100 was added to a final concentration of 1%. After 10 min, the nuclei were sedimented by centrifugation at 2,500 r.p.m. for 10 min (SS34 rotor), washed in buffer 2 without Triton and suspended in 2.2 M sucrose, 1 mM $MgCl_2$, 0.1 mM dithiothreitol and 1 mM PMSF. Centrifugation at 40,000*g* for 1 h in the SW27 (Beckman) sedimented the clean nuclei to the bottom of the centrifuge tube. The purified nuclei were washed twice with 0.14 M NaCl, 0.01 M Tris at *p*H 7.5 before extracting them three times for 1 h each with 0.4 N H_2SO_4 at 4 °C. The histones were precipitated from the combined supernatants by addition of 5 volumes of 95% ethanol and placed at −20 °C overnight. Each histone class in the Triton gels was identified by two methods. (1) Individual histone fractions were extracted by a selective chemical extraction described by Johns[22]. The purity of the fractions was checked by their separations on acid–urea gels[15]. The same individual histone samples were then separated on the Triton–acid–urea gels and their migration was identified. (2) Large diameter Triton gels were prepared on which 1 mg of protein was separated. The gels were sliced into 2-mm sections from which the histones were extracted overnight in 0.15 M NaCl. The location of each histone was determined by assaying each fraction with individual anti-histone antisera in the complement fixation assay described by Stollar and Ward[23]. For this figure, 25 µg of histone was separated on 12% polyacrylamide gel containing 8 M urea and 6 mM Triton X-100[16,17]. Bands 1 and 2 are $f2a2_1$ and $f2a2_2$, respectively. The gels were stained with 0.4% Amido Black and acetic acid : methanol : water (1 : 5 : 14) and scanned at 570 nm with a spectrophotometer (Beckman).



Many different DMSO-responsive cell lines of Friend leukaemia, including the original 745 line from C. Friend, have this altered f2a2. The change in the ratio of the f2a2 subfractions seems to be a prerequisite for the ability of the cells to respond to DMSO. The f2a2 ratio does not change when the cells respond to DMSO.

With increasing time in culture, previously unresponsive cell lines began to respond to DMSO and to show an increase in $f2a2_2$ and concomitant decrease in $f2a2_1$. An uncloned cell line, TP46, established from passage 46 of a leukaemic subcutaneous tumour, was unresponsive to DMSO and retained an $f2a2_1 : f2a2_2$ ratio of 2 : 1 when examined at passages 5, 10 and 18. At passage 36, 10 weeks later, however, the cell line responded to 1.5% DMSO and demonstrated an increase in $f2a2_2$ (Fig. 2). Four cloned cell lines, C7A→D, showing different relative responses to DMSO, were derived from another cloned cell line, C7, established from passage 25 of uncloned line TP32. C7 was initially unresponsive to DMSO but began to show a response after several months' passage in tissue culture. Clone C7C did not synthesise detectable levels of haemoglobin when cultured in 1–2% DMSO. In contrast, C7D responded well to small amounts of DMSO. C7A and C7B were intermediate in their response. Morphologically, all cell lines were similar. Analysis of the histones in these cell lines showed a direct correlation

Table 1 Percentage of each f2a2 subfraction in the total f2a2

	DMSO response	% f2a2 Histone in total histone	% Subfractions in total f2a2		
			$f2a2_1$	$f2a2_2$	$f2a2_1/f2a2_2$
Normal spleen cells		22	75	25	3.0
Normal liver cells		18	75	25	3.0
Phenylhydrazine-treated mouse spleen cells		21	78	22	3.5
21-d Leukaemic spleen cells		17	77	23	3.3
Tumour cells (passage 40, *in vivo*)		21	77	23	3.3
Uncloned cell lines					
TP46P18	−	18	70	30	2.3
TP46P39	++++	22	50	50	1.0
TP63P8	−	17	66	34	1.9
Cloned cell lines					
C7A p8	++	20	60	40	1.4
C7B p8	++	21	63	37	1.6
C7C p7	±	17	66	34	1.9
C7C p27	++	20	62	38	1.5
C7D p8	++++	21	47	53	0.8
F1* p50	+++	20	55	45	1.2
C17 p10	++++	19	44	56	0.8
Tumour from C17 cells	++++	19	47	53	0.9

Histones were separated on 12% polyacrylamide gels containing 8 M urea and 6 mM Triton X-100. The percentage of each f2a2 subfraction was determined by weighing photocopy paper of each histone peak from a densitometer tracing and dividing that by the combined weight of both subfractions. The amount of bound dye was directly proportional to the amount of protein in each band (< 20 µg) (ref. 20). TP, tumour passage; +, relative amount of haemoglobin produced in response to DMSO.

* Original line 745.

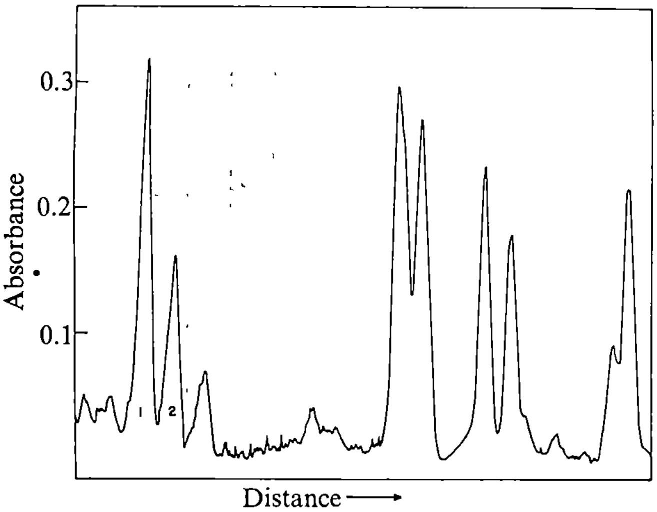


Fig. 2 Histones from uncloned cell line (TP46P18) were extracted and separated as described in the legend to Fig. 1.

between the amount of haemoglobin synthesised in response to DMSO and the amount of $f2a2_2$ (Fig. 3). The results from C7A and C7B may represent an average from responsive and unresponsive cells now present in these originally cloned lines or a decreased DMSO response in all cells. The fact that both types of cells were cloned from the original cloned C7 line suggests that with time in tissue culture a change towards inducibility occurs in some, but not all, cells.

f2a2 Histone is a major component of chromatin representing 20% of the histones of all mouse cells tested including both inducible and non-inducible cell lines. The changes observed in f2a2 subfractions are suggestive of either (1) a chemical modification of the $f2a2_1$ fraction causing it to migrate in a different position in the gel, that is, $f2a2_2$, or (2) a change in the synthesis of the two f2a2 subtypes similar to the situation found during sea urchin development[18]. The strong association of this histone change with the ability of the cell to respond to DMSO suggests that the change opens up groups of genes which can now be induced by DMSO. Evidence from different laboratories indicates that DMSO affects both physical structure and biochemical function of chromatin[12,13]. In the Friend cell system, DMSO produces a group of changes related to erythroid maturation[6–10]. This pleiotropic response may be explained by (1) a structural change in the chromatin of the already committed erythroid cell produced by the $f2a2_2$ modification and (2) a further change in this altered chromatin by DMSO.

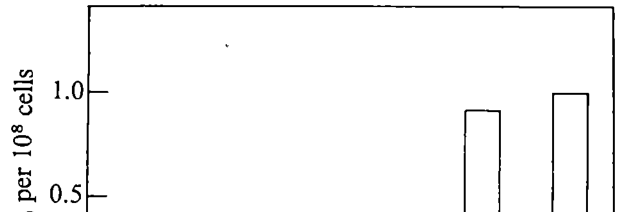


Fig. 3 Relationship of haemoglobin production to relative amounts of $f2a2_2$. After 5 d in 1.5% DMSO, cells were counted, washed twice in 0.14 M NaCl and separated into two fractions. One fraction was placed in distilled water and the cells were disrupted in 1% sodium dodecyl sulphate. Cell debris was removed by centrifugation at 1,500*g* for 10 min and the supernatant solution was read at 410 nm (refs 20 and 21). From the second fraction the histones were extracted (Fig. 1) and the amount of each f2a2 subfraction was determined.

Histones extracted from leukaemic spleens and erythroid spleens do not show the increase in $f2a2_2$ seen in DMSO-responsive Friend cell lines, although haemoglobin synthesis occurs. This may reflect the heterogeneity in the cells taken from these spleens. Studies of early erythroid cells isolated from these tissues are in progress to resolve this question. Whatever differences may exist between the control of erythroid development *in vitro* and *in vivo* seem to be circumvented by DMSO in the presence of an alteration of f2a2, since the contribution of two factors leads to apparently normal erythroid maturation[6–10].

The fact that histone f2a2 is so conserved during evolution suggests that the specific change we observe in the ratio of the f2a2 subfractions serves a purpose that enables the Friend cells to grow autonomously in tissue culture and to respond to DMSO *in vitro*.

We would like to thank D. B. Stollar, of Tufts Medical School, for his assistance with the complement fixation assay and L. Cohen and C. Alfagime, of the Institute for Cancer Research, for their helpful discussions.

This work was supported in part by a grant from the National Leukemia Association, a fellowship of the Leukemia Society of America (L.A.B.) and a research career development award (S.B.L.).

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Received November 28, 1975, accepted February 19, 1976

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DNA ligases during rat liver regeneration

DNA ligase catalyses the formation of phosphodiester bonds at single-strand breaks in double-stranded DNA[1]. Since DNA is replicated discontinuously, and radiation-induced DNA single-strand breaks are effectively repaired in both bacteria and eukaryotes, bacteria and mammalian cells will require DNA ligase function both for replication and repair of DNA[2–5]. A DNA ligase activity has also been found in and purified from mammalian cell extracts[6]. It required ATP as a cofactor and was found in highest amounts in tissues containing rapidly dividing cells. In agreement with these data it has been reported that the DNA ligase activity increased five- to tenfold in rat liver extracts during liver regeneration[7,8]. We have shown that mammalian cells contain two different DNA ligases (I and II) both of which are found in nuclei and cytoplasm, but neither of which is predominantly a mitochondrial enzyme[9,10]. Both enzymes require ATP but have different molecular weights, fractionation properties, and heat stability. Furthermore, rabbit antibodies against purified calf thymus DNA ligase I effectively inhibit DNA ligase I from calf, mouse, rabbit, and human tissues, whereas DNA ligase II from the same sources is not inhibited. It was therfore of interest to determine whether the increase in total DNA ligase activity reported to occur during liver regeneration was due to an increase in both DNA ligase I and DNA ligase II activities or due to an increase in only one of the two enzymes. Such determinations are now possible when chromatographic properties of the enzymes are known, and antibodies against DNA ligase I are available. This report shows that the level of DNA ligase I activity increases about 15-fold during rat liver regeneration, whereas the level of DNA ligase II activity remains essentially unchanged.

Male Sprague–Dawley rats (~200 g) were partially hepatectomised (to about 70%) as described previously[11]. At indicated times after hepatectomy two rats were killed, the livers immediately taken out, rinsed in ice-cold 0.25 M sucrose, rapidly frozen to −70 °C, and kept at this temperature until preparation. Cell extracts from equal amounts of liver from two rats (approximately two half livers) were prepared and purified by streptomycin treatment, ammonium sulphate fractionation (40–65% saturation), and stepwise phosphocellulose chromatography as described previously[10]. These fractionation steps were used to remove nucleases and other activities potentially interfering in the enzyme assays. Earlier experiments with calf tissue DNA ligases have not given indications that any of the two DNA ligases are selectively lost during these purification steps[10]. DNA ligases I and II were separated by hydroxyapatite chromatography: I activity was eluted with 0.14 M potassium phosphate (*p*H 7.2) and II with 0.40 M potassium phosphate[10]. Assignment of these fractions with DNA ligase I and II were verified by experiments with the anti-serum against calf thymus DNA ligase I, which inhibited more than 95% of the ligase activity in the 0.14 M eluate, whereas the activity in the 0.40-M eluate was inhibited 0–20% due to tailing of DNA ligase I during chromatography, as described previously[10]. The eluted hydroxyapatite fractions were pooled, concentrated by ammonium sulphate precipitation, redissolved, and dialysed against 0.3 M NaCl–0.05 M Tris-HCl–1 mM EDTA–1 mM dithiothreitol (*p*H 7.4) for 14–16 h before assay. DNA ligase activity was determined after preincubation of the fractions at 0 °C for 5 min with either control serum or antiserum against DNA ligase I. The ligase assay used measures the conversion of 5′-^{32}P-phosphoryl termini at single-strand breaks in double-stranded DNA to a form resistant to alkaline phosphatase[10,12].

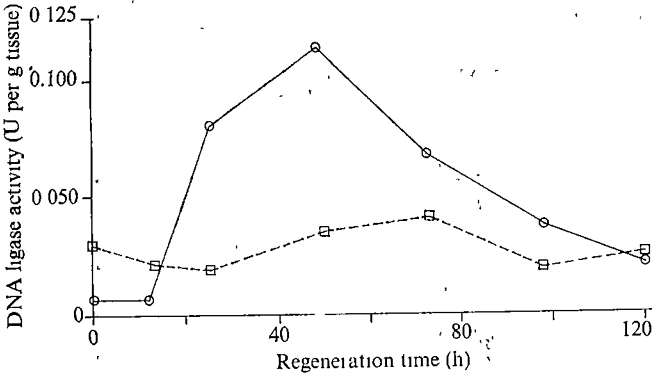

Fig. 1 Levels of activity of DNA ligases I (○) and II (□) during rat liver regeneration Each point represents duplicate experiments with several different enzyme concentrations.

The data obtained for the different time points are given in Fig. 1. Normal rat livers contain a relatively low level of DNA ligase activity, and most of this activity is due to DNA ligase II. The levels of this enzyme seem to remain essentially constant during liver regeneration as the measured variation is less than twofold. In contrast, DNA ligase I activity increases approximately 15-fold to a broad maximum at about 48 h regeneration time; and is thus the dominant DNA ligase activity in extracts from regenerating rat livers. After 5 d regeneration, the level of DNA ligase I had almost returned to the value found in normal rat liver. It is clear that the previously discovered increase in DNA ligase activity during liver regeneration[7,8] is almost entirely due to an increase in DNA ligase I. Together with our earlier published results[9,10], the present data strongly indicate that mammalian cells contain two different DNA ligases.

The isolated hydroxyapatite fractions of DNA ligases I and II from the 48-h regenerating livers were used for two additional control experiments. When separately chromatographed on Sephadex G-150 in 0.3 M NaCl–0.05 M Tris-HCl–1 mM EDTA–1 mM dithiothreitol *p*H (7.4), the ligase activity of these fractions showed identical elution properties to the corresponding calf thymus enzymes. Furthermore, when the separate enzyme fractions were heated at 45 °C for 5 min in the same buffer as that used for gel chromatography (protein concentration: 3 mg ml^{-1}), the rat DNA ligase I fraction retained more than 95% of its initial activity, whereas the rat DNA ligase II retained less than 5% of the initial activity. These data show that the rat DNA ligases are of similar heat lability and molecular weight as the corresponding calf thymus enzymes. To determine whether the apparent increase in DNA ligase I activity could be explained by the presence of DNA ligase I inhibitors in the 0-h liver fractions, or by the presence of effectors in the 48-h fractions, mixing experiments were carried out. Calf thymus DNA ligase I (1×10^{-3} U 1,000-fold purified enzyme) was separately mixed with several concentrations (0.5–3×10^{-4} U) of the 0-h or 48-h hydroxyapatite fractions of rat liver DNA ligase I. In all cases, the observed DNA ligase activity was identical (±10%) to the expected value calculated as the sum of the individual ligase activities of the mixed fractions. Thus, the 15-fold increment in DNA ligase activity during liver regeneration did not seem to be due to artefacts such as differences in interfering factors between the extracts. It is clear that whereas DNA ligase I activity in normal rat liver is low, the cells still contain detectable amounts of this enzyme. Similar control experiments with fractions containing DNA ligase II also demonstrated the absence of assay artefacts in this case. To check liver regeneration, the amount of DNA polymerase α activity was measured in the cell extracts, and was about five times higher after 24 h regeneration than in extracts from control livers, in agreement with earlier determinations[13]. Moreover, the weight of the livers successively increased during regeneration.

Regenerating liver has often been used as a system in which a population of non-dividing (G_0) cells is turned into an actively proliferating state with a high level of DNA replication. Many enzymes associated with synthesis of DNA and its precursors are known to increase during liver regeneration, but the time required for maximal induction varies considerably for the different enzymes. Moreover, in the case of multiple enzymes with similar function, the response of the individual enzyme activities to regeneration might be quite different. In the case of mammalian DNA polymerases, the DNA polymerase α activity shows a five- to sevenfold increase during rat liver regeneration[13], and DNA polymerase γ activity has also been reported to increase in cells actively synthesising DNA[14]. In contrast, the level of DNA polymerase β activity remains constant or shows only a minor increase in the same conditions[13,14]. The increase in DNA polymerase α and γ during cell proliferation in these and other systems has been considered as circumstantial evidence for their involvement in DNA replication[13,14]. The present data on DNA ligases I and II levels during liver regeneration clearly resemble the previous findings for the DNA polymerases. In this regard, note that DNA ligase I has also been found in relatively large amounts in rapidly dividing cell populations such as calf thymus and mouse ascites tumour cells, but in much lower amounts in calf liver; whereas DNA ligase II levels have shown relatively minor variations between different tissues[10]. As the levels of both DNA polymerase α and DNA polymerase γ activities increase during cell proliferation, it seems possible that DNA ligase I acts together with one or both of these enzymes in DNA replication, and that the cellular levels of that polymerase and the ligase are regulated in coordination. DNA polymerase β and DNA ligase II, neither of which show increased levels during cell proliferation, may then have functions in maintaining the integrity of DNA.

I thank Dr Tomas Lindahl for discussions, Dr Bernd Otto for carrying out the measurements of DNA polymerase α activity, and Ms Berit Sperens for assistance. This work was supported by the Swedish Natural Science Research Council, the Swedish Cancer Society and the Karolinska Institutet.

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Single-stranded regions in regenerating rat liver DNA

SEVERAL groups have identified single-stranded regions in actively replicating eukaryote DNA[1–5]. We have used isopycnic caesium sulphate gradients containing silver ion, and a single-strand-specific endonuclease to determine the single strandedness of DNA from actively regenerating rat liver, and from 'control' liver of partially hepatectomised rats that had been allowed to regenerate their normal liver mass. More single-stranded DNA was observed in regenerating liver than in control livers.

One-month-old male Sprague–Dawley rats were subjected to partial hepatectomy[6]. Seven animals were killed 20.5 h later and their livers were removed. A second group of seven was housed and fed *ad libitum* for 14 d before being killed; in these conditions more than 95% of the original liver mass was restored[7]. Nuclei were isolated by the method of Blobel and Potter[8] and DNA was purified using Marmur's procedure[9].

Figure 1 shows the results of Cs_2SO_4–Ag^+ isopycnic centrifugation of DNA from the two groups of hepatectomised rats. The gradient profile of a mixture of native and heat-denatured 14-d hepatectomised rat DNA is given in Fig. 1*a*. Denaturation of DNA causes an increase in silver ion binding leading to an increase in buoyant density[10]. Denatured DNA banded with a peak density of 1.63 g cm^{-3} and native DNA banded with a peak density of 1.50 g cm^{-3}. The identities of the peaks were confirmed when native DNA was centrifuged separately (data not shown). When DNA from actively regenerating (20.5-h) liver was centrifuged in identical conditions (Fig. 1*b*), its buoyant density was greater than that of 14-d regenerating DNA by 0.09 g cm^{-3} (Fig. 1*a*). After dialysis against 0.015 M NaCl–0.0015 M sodium citrate, *p*H 7.5, the peak fraction of Fig. 1*b* had a characteristically double-stranded thermal denaturation pattern and a hyperchromicity of 37%. (It should be noted, however, that thermal denaturation patterns detect only large amounts of single-strandedness.) Thus, DNA from actively proliferating liver seems to be more single-stranded than DNA from control liver.

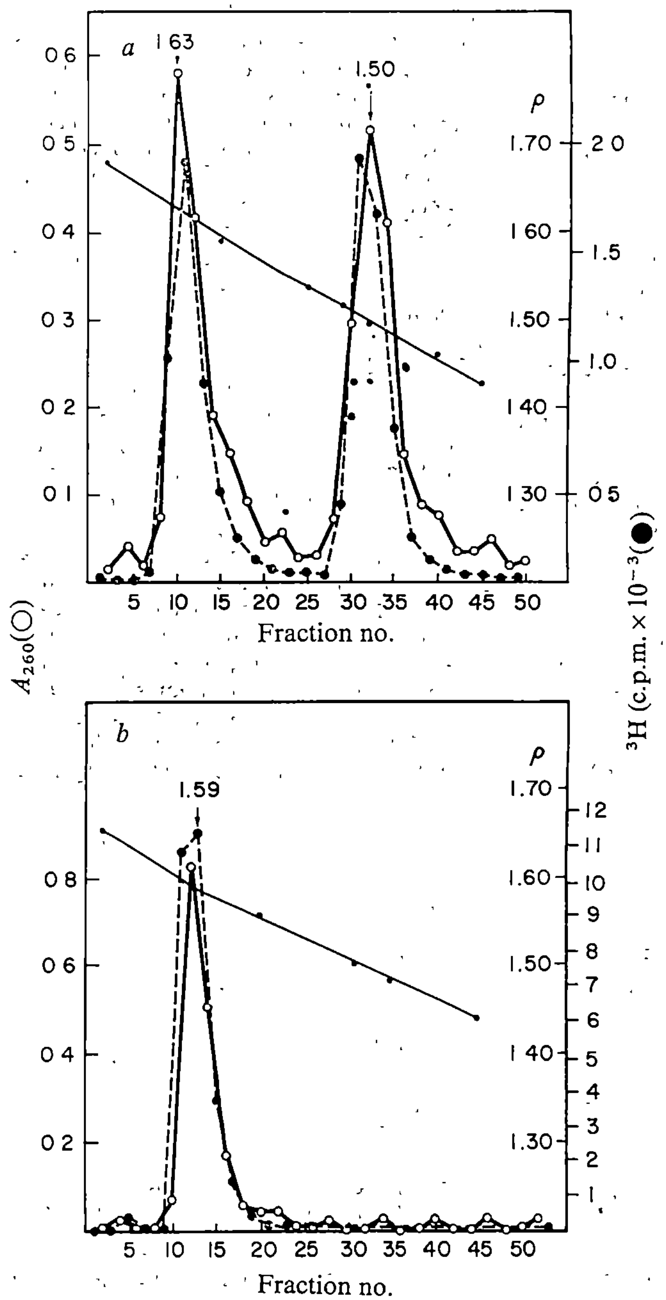

Fig. 1 Cs_2SO_4–Ag^+ isopycnic centrifugation of 20.5-h and 14-d regenerating rat liver DNA. The procedure has been described before[10]. Rats were injected 20 h after hepatectomy, through the tail vein, with ^{3}H-methyl thymidine (New England Nuclear; 50.8 Ci mmol^{-1}) at a concentration of 1 μCi per body weight. Specific activities of the 20.5-h and 14-d hepatectomised rat liver DNAs were 780 c.p.m. μg^{-1} and 127 c.p.m. μg^{-1}, respectively. DNA was dialysed sequentially against 0.1 M EDTA, *p*H 8, 0.1 M $NaClO_4$ and 0.01 M Na_2SO_4. The DNA (200 μg) was added to 6.3 g Cs_2SO_4 (Gallard–Schlesinger ultrapure) in sodium borate buffer, *p*H 9.2, to give a final concentration of 5mM $AgClO_4$ was added in drops to a final concentration of 16 μM and a molar ratio of Ag–DNA-P of 0.27. The solution was adjusted to a density of 1.5 g cm^{-3} and a final volume of 10 ml by the addition of water and/or Cs_2SO_4. Centrifugation was performed in a Beckman model L3-50 ultracentrifuge in a type 65 rotor at 20 °C for 70 h at 35,000 r.p.m. Fractions were collected from the bottom. Densities of samples of selected fractions were calculated from refractive indices determined with a Bausch and Lomb refractometer. Samples (0.1 ml) of odd-numbered fractions were applied to filter disks, dried, and washed sequentially in cold 5% trichloroacetic acid, ethanol, and ether. The radioactivity was determined in a Liquifluor–toluene cocktail with a Beckman LS-355 liquid scintillation counter at an efficiency of 54% for tritium. Even-numbered fractions were diluted with 0.5 ml of water and the A_{260} was determined with a Gilford model 2400-2 spectrophotometer. *a*, 100 μg heat-denatured DNA plus 100 μg native DNA from 14-d regenerating rat livers. *b*, 200 μg native 20.5-h regenerating rat liver DNA.

The kinetics of release of acid-soluble material from single-stranded DNA by S1 nuclease from *Aspergillus oryzae*[11] was determined using heat-denatured calf thymus DNA. After 30 min of treatment with S1 nuclease, approximately 90% of denatured DNA was rendered acid-soluble. To test for the presence of endogenous nucleases in our rat liver DNA preparations, the DNA was incubated without added enzyme. No significant release of acid-soluble material was detected.

To confirm that our S1 nuclease preparation was free of contaminating double-strand nuclease activity, a covalently closed circular DNA (R6K plasmid[12]) was examined by sedimentation in sucrose density gradients before and after treatment with S1 nuclease (data not shown). The only action observed was a slow nicking of the supercoiled covalently closed circular form. A similar slow nicking of another covalently closed circular molecule (φX174 RFI) by S1 nuclease has been reported by Godson[13]. Thus, our nuclease seems to be free of double-strand nuclease activity.

DNA from each group of rats was treated with S1 nuclease. The quantities of A_{260} U of 20.5-h and 14-d post-hepatectomy DNAs that were sensitive to S1 nuclease were 3.9% and 1.3%, respectively. Thus, DNA obtained 20.5 h after hepatectomy contained three times more single-strand nuclease-sensitive sites than DNA from 14-d regenerate livers. Since the mitotic index of cells from actively regenerating liver is about 300 times higher than that of cells from normal liver[14], our results seem to correlate with cellular replication. Our findings agree with those of Suzuki, *et al.*[15], who, using the electron microscopic immunoperoxidase method, found more single-stranded sites in DNA in nuclei from regenerating liver than in nuclei from control liver. DNA from actively replicating cells of the iris of the newt, *Triturus*, has been reported to contain more single-stranded regions than DNA from non-replicating cells[5].

In summary, there seem to be more single-stranded regions in DNA from actively dividing liver cells than in DNA from non-dividing liver cells. The physiological significance of single-stranded regions is not clear, but they are likely to be involved in DNA replication. This work was supported by grants from the National Cancer Institute and the American Cancer Society. We thank Dr Francis L. Macrina for R6K DNA.

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Nalidixic acid and bacterial chromosome replication

NALIDIXIC acid (NAL) has been described as a specific inhibitor of bacterial DNA synthesis *in vivo* and *in vitro*[1–3], but its mechanism of action on susceptible bacteria remains obscure. There is no evidence to indicate that NAL can bind to DNA[3,4], and none of the enzymes known to be involved in DNA replication is affected by NAL *in vitro*[3–6]. But it is known that *nalA* mutants of *Escherichia coli* are resistant to the drug[7], as are *in vitro* DNA replicative systems derived from such mutants[3]. This suggested that a comparative study of DNA replication in *nalA* mutants and otherwise isogenic bacteria should facilitate identification of the hitherto unknown NAL target. We report here that NAL specifically arrests the later stages of chromosomal replication in bacteria sensitive to the drug.

Continuous DNA-labelling with ^{3}H-thymidine was done as before[8] using *E. coli* strains KL16 (NAL^{s}) and MH5 (a *nalA* mutant of KL16; ref. 7); in the presence and absence of the drug at 20 μg ml^{-1}. The results (Fig. 1) show that addition of NAL rapidly inhibited DNA synthesis in strain KL16 without significantly affecting that of strain MH5.

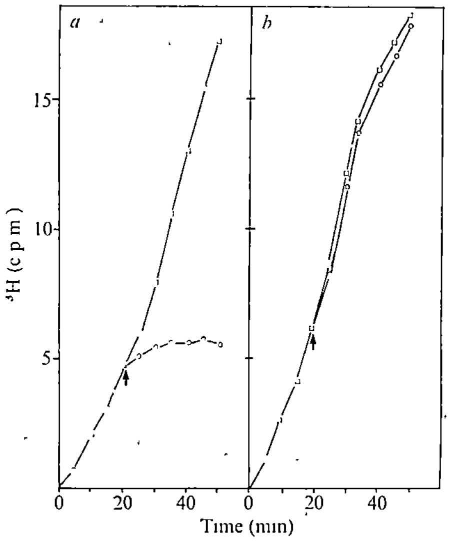
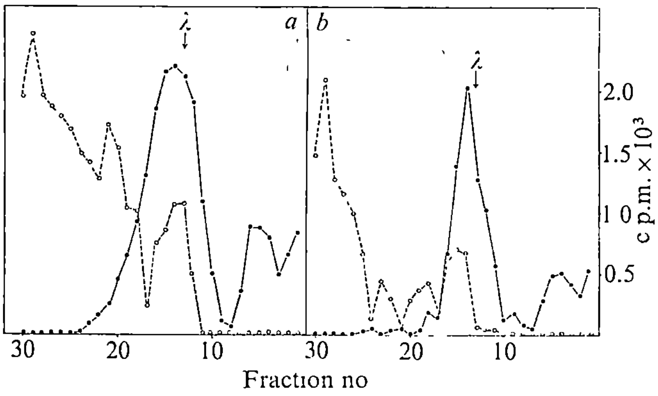


Fig. 1 Incorporation of ^{3}H-thymidine into DNA measured by continuous labelling in the presence and absence of NAL at 20 μg ml^{-1}. *a*, *E. coli* KL16; *b*, *E. coli* MH5. For both strains: □, no drug; ○, drug-treated. The arrows indicate the time of addition of NAL to 20 μg ml^{-1}. Bacteria (initially 10^{7} viable units per ml) were cultured at 30 °C in Davis and Mingioli (DM) medium containing ^{3}H-thymidine (0.1 μCi ml^{-1}) and deoxyadenosine (50 μg ml^{-1})[8]. At the times shown 1-ml samples were treated with 0 1 ml trichloroacetic acid at 0 °C and filtered through Whatman GF/C filters After washing with 1 volume of 1% trichloroacetic acid, boiling distilled water (3 volumes), 5% NaCl (1 volume) and 5% acetic acid (2 volumes), the filters were dried and radioactivity was determined by scintillation counting.

Pulse labelling[9,10], however, yielded markedly different results Using alkaline sucrose density gradient centrifugation with a 5-min pulse of ^{3}H-thymidine and a 15-min label-free chase period, DNA replication in the two strains was examined both in the absence of the drug and after 20 min of treatment with NAL (20 μg ml^{-1}). Monomeric linear ^{14}C-labelled phage λ DNA was used as a sedimentation marker throughout. In the untreated cells of strain KL16 an even distribution of single-stranded DNA fragments was observed throughout the gradient after pulse-labelling (Fig. 2*a*) During a subsequent label-free chase period all the low and most intermediate sized DNA precursors had been converted into DNA of high molecular weight. When the bacteria were treated with NAL for 20 min before a pulse label, single-stranded DNA fragments were still synthesised (Fig 2*b*). DNA of high molecular weight, however, was absent and there was an abnormal accumulation of DNA of intermediate size of about 38S. Moreover, these 38S DNA precursors could not be chased into DNA of higher molecular weight.

When the amount of pulse label incorporated into DNA

Fig. 2 DNA size distributions. *a*, Distribution of pulse-labelled (●) DNA from strain KL16 obtained by alkaline sucrose gradient centrifugation: ○, results obtained after a similar pulse followed by a 15 min label-free chase period. *b*, Distribution of pulse-labelled (●) DNA from strain KL16 obtained by alkaline sucrose gradient centrifugation of a culture treated with NAL (20 μg ml^{-1}) for 20 min before isolation: ○, results obtained after a similar pulse followed by a 15-min label-free chase period in the presence of NAL. (Fractions are numbered from the top of the gradient.) Bacteria (approximately 10^7 viable units per ml) were inoculated into 10 ml DM medium at 30 °C. Thymidine and deoxyadenosine were added to give final concentrations of 200 μCi ml^{-1} and 50 μg ml^{-1}, respectively. After 5 min, half the culture was membrane-filtered and washed with 2 volumes of chilled medium lacking glucose, deoxyadenosine and thymidine (pulse). The other half of the culture was also filtered after 5 min but was washed with 2 volumes of DM medium lacking deoxyadenosine and thymidine at 30 °C. This filter membrane was immersed in 5 ml of fresh DM medium lacking only deoxyadenosine and thymidine at 30 °C, the cells were detached from it by agitation and the membrane discarded. After 15 min at 30 °C the cells were collected again by membrane filtration and washed with 2 volumes of chilled medium lacking glucose, deoxyadenosine and thymidine (pulse chase). The two membranes carrying pulsed and pulse-chased bacteria were resuspended in 1 volume of DM medium lacking glucose, deoxyadenosine and thymidine and the bacteria were detached by agitation. The membranes were discarded and the suspension was centrifuged at 3,000*g* for 10 min. The pellets were each resuspended in 0.6 ml of 0.1 M NaOH containing 10 mM EDTA. λ phage DNA labelled with ^{14}C was added as a 34S sedimentation marker and the mixture was incubated at 37 °C for 1 h. After clarification at 3,000*g* for 10 min, the upper 0.4 ml of each supernatant was layered on 13 ml sucrose gradients (5–20%) of 0.9 M NaCl, 0.1 M, NaOH and 2 mM EDTA and centrifuged at 38,000 r.p.m. in an S40/135 rotor for 3 h at 20 °C. Thirty fractions were then collected from the top of the gradient and 1 ml of cold 10% trichloroacetic acid added to each. Precipitates were collected on Whatman GF/C filter disks and washed with 5×10 ml portions of cold 1 M HCl. The filters were dried and radioactivity determined by scintillation counting.



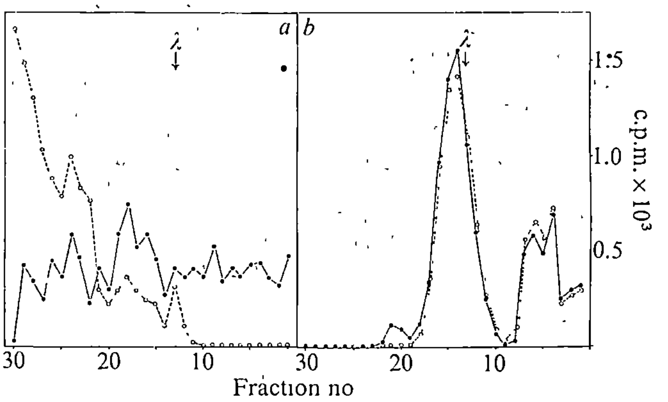


Fig. 3 DNA size distributions. *a*, Distribution of pulse-labelled DNA (●) from strain MH5 obtained by alkaline sucrose density gradient centrifugation; ○, results obtained after a similar pulse followed by a 15 min label-free chase period *b*: ●, DNA from strain MH5 obtained by alkaline sucrose density gradient centrifugation of a culture treated with NAL (20 μg ml^{-1}) for 20 min before pulse labelling; ○, results obtained after a similar pulse followed by a 15-min label-free chase period in the presence of NAL. (Fractions numbered from the top of the gradient.) The experimental details were the same as those described in the legend to Fig. 2.

in the presence and absence of NAL was calculated, the result was surprising. NAL (20 μg ml^{-1}) inhibited DNA synthesis by only 14% after 20 min of treatment, whereas the continuous label study had indicated that DNA synthesis was inhibited by about 80% in identical conditions.

Examination of the DNA replication of the *nalA* mutant strain MH5 by pulse labelling showed that this organism accumulated 38S single-stranded DNA fragments even in the absence of the drug (Fig. 3*a*) During the chase period these precursors were converted slowly into DNA of high molecular weight. When the experiment was repeated with strain MH5 treated with NAL (20 μg ml^{-1}) for 20 min before a pulse label, the accumulation of DNA precursors and their conversion into DNA of high molecular weight proceeded relatively unimpaired (Fig. 3*b*).

Thus the *nalA* mutation slows the joining of 38S DNA precursors and sensitive bacteria treated with NAL are inhibited at the same step, in that they accumulate 38S DNA fragments which cannot be converted into DNA of higher molecular weight. Thus it seems that the *nalA* gene product has a unique role in the sealing of 38S single-stranded DNA precursors, which seems to be a hitherto unknown vital step in the replication of the bacterial chromosome.

There remains the apparent discrepancy between the continuous and pulse labelling results with strain KL16. When the sequential events of DNA replication are considered, however, the two results can be reconciled as follows.

In bacteria sensitive to NAL, exogenous ^{3}H-thymidine is incorporated into 10S Okazaki fragments[11]. These are joined by DNA ligase into longer single-stranded DNA, of molecular weight 38S, which in turn are converted into full-sized daughter molecules. This last step seems to be governed by the action of the *nalA* gene product. When sensitive bacteria are treated with NAL it is only this step that is inhibited (Fig. 2) so that gaps remain between each 38S single-stranded DNA precursor. Thus NAL does not inhibit the incorporation of ^{3}H-thymidine into trichloroacetic acid insoluble single-stranded DNA of low or intermediate molecular weight, as measured by pulse labelling. In other words, NAL does not inhibit DNA synthesis. But, the newly synthesised single-stranded DNA precursors, by virtue of the unsealed gaps, are most probably metabolically unstable, as they will be substrates for exonuclease action. Such degradation, which has been described during NAL treatment of *Bacillus subtilis*[14], would return the ^{3}H-label to the intracellular thymine pool from where it would be recycled into Okazaki fragments. Thus net DNA synthesis would cease because of the transience of the newly synthesised DNA intermediates, and this is the result we obtained with continuous labelling of DNA. Experimental support for these views is given by the finding that competent RNA and protein syntheses are essential prerequisites for both exonuclease action and the bactericidal action of NAL[8,13], and thus our results seem not only to provide evidence about the nature of the target site for NAL, but also to indicate how the drug kills bacteria.

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π Turn is a conformational pattern in RNA loops and bends

A PATTERN has been found in the structure of transfer RNA by which a polynucleotide chain can make an abrupt change in direction so as to make a sharp cornered loop or a sharp bend.

Nucleotide sequences[1] of transfer RNAs (tRNAs), ribosomal RNAs (rRNAs) and phage RNAs suggest that they can be represented as a series of double-stranded helices with loops separated by short stretches of single-stranded RNA. The single-stranded loops and short stretches have been implicated as the recognition sites for proteins or other nucleic acids.

The three-dimensional structure of yeast tRNA^{Phe} determined by X-ray crystallography provides conformational information about such single-stranded regions. The backbone structure of this tRNA molecule revealed that the loops have sharp corners[2] rather than being smooth. This feature was verified in detail at 3-Å resolution as well as at higher resolution for the same tRNA in two different crystalline lattices[3–7]. Examination of the TψC and the anticodon loops, both with seven nucleotides, shows that the way the backbone makes a sharp turn is the same for both loops, suggesting that this is a pattern of forming a sharp cornered loop (Fig. 1). The reversing of

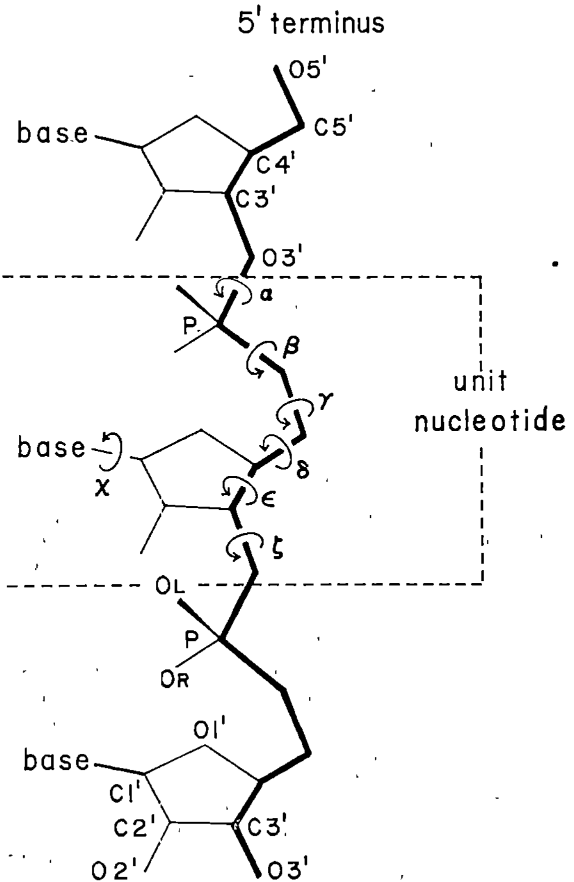

Fig. 2 The nomenclature of torsional bonds used here is a modification from that suggested in ref. 9 to accommodate all the torsional angles in one 'unit nucleotide'.

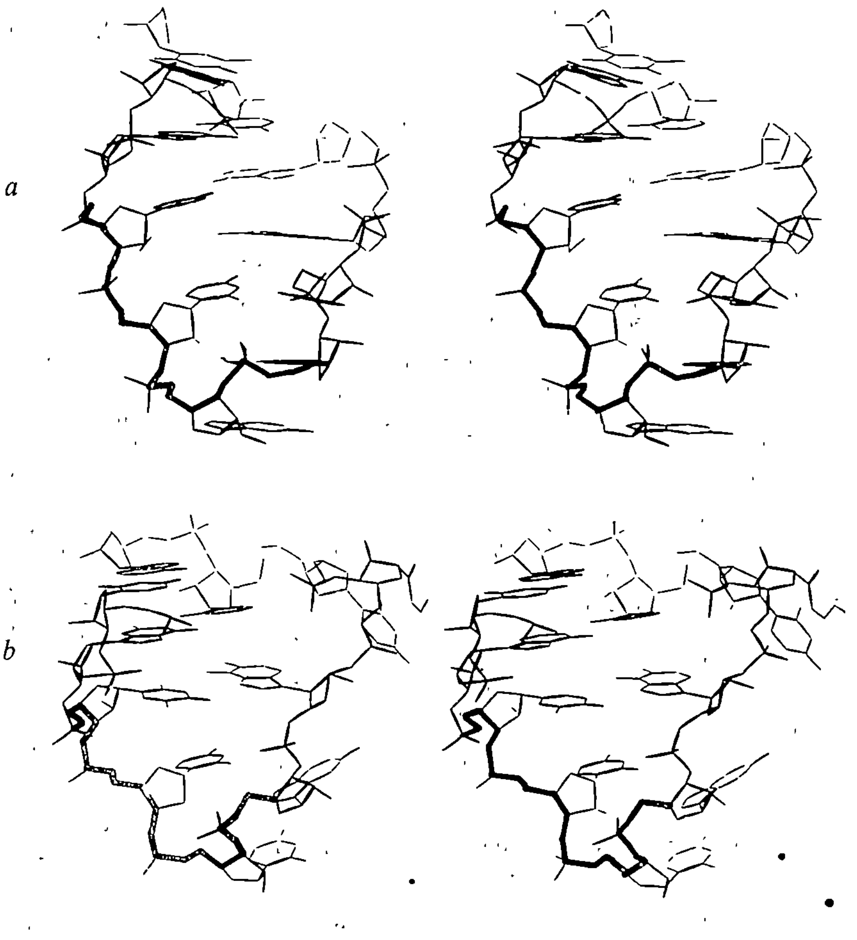

Fig. 1 Stereo view of the anticodon loop (*a*) and the TψC loop (*b*) of yeast tRNA^{Phe} At the top of each figure are two base pairs just before the loop. The 5′ end is at the upper left and the 3′ end at the upper right. The π turn occurs at the phosphodiester link between the bottom nucleoside and that at the 5′ side of it. These computer drawings are based on the atomic coordinates of this tRNA structure in the orthorhombic crystal form[6] The side chain tail of the Y base has been omitted from (*a*) for clarity, and the backbone of the 5′ half of the loops are darkened.

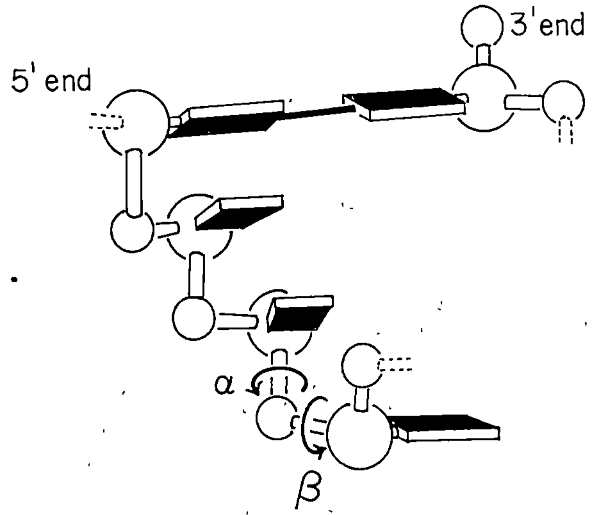

Fig. 3 Schematic drawing of the common portion of Fig. 1*a* and *b*. The circular arrows indicate two bonds involved in π turn to reverse the direction of the backbone from a helical conformation.

the direction of the backbone chain from the helical conformation occurs, in both cases, within just one phosphodiester link by rotating primarily around O3′–P and P–O5′ (torsion angles α and β, see Fig. 2) of the third nucleotide from the 5′ end of the loop. These two phosphoester bonds were initially pointed out to be the two least constrained backbone torsion angles[8]. This is shown schematically in Fig. 3. Besides these two examples there are several more sharp turns formed by similar rotations around the α and β in the DHU loop, the variable loop, between the helices and in the TψC loop.

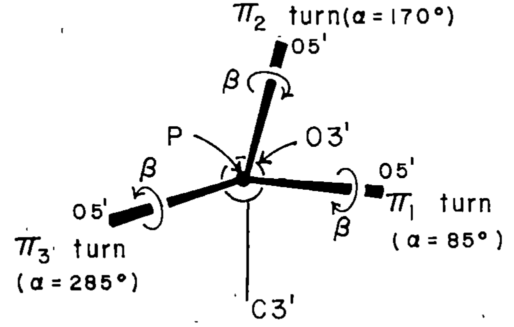

Fig. 4 Three families of π turns, as distinguished by their α angles, are viewed along the P–O3′ bond. The sharpness of the turn is dictated by how much the β angle deviates from a helical conformation.

For convenience, we define the π turn to describe a set of sharp turns in a polynucleotide chain, all due to the rotation around two adjacent phosphoester bonds, causing changes in the direction of the backbone chain. There are three possible families of π turns, depending on the orientation of the P–O5′ bond with respect to the C3′–O3′ bond, that is the torsion angle α, as shown in Fig. 4: called π_1, π_2 and π_3 turns with α values near 85°, 170° and 285° respectively. The three representative α angles chosen here are based on the observed values in crystal structures of several dinucleoside phosphates[9]. The most common turn found in yeast tRNA^{Phe} is the π_3 turn. This is observed in the phosphodiester links between residues 9 and 10, 17 and 18, 33 and 34, 46 and 47 and 55 and 56. The sharpness of the turn depends on how much the β angle deviates from a helical conformation (near 290°) (ref. 9). The other two classes of π turns are also found in this tRNA structure; for example, π_1 turn between 47 and 48; π_2-turn between 15 and 16. There are several more π turns in the tRNA structure which are uncertain, at the current stage of refinement, to assign a specific class.

The π_1 turn has been observed in the crystal structures of uridylyl 3′,5′-adenosine phosphate, UpA[10,11], ApApA[12] and ApU·9-aminoacridine[13] where two oppositely oriented riboses are connected by one phosphodiester linkage.

Thus, it seems that the π turn provides a simple, general pattern by which a polynucleotide chain makes a sharp turn, either to form a loop or to change the direction of the chain flow. Such a sharp cornered loop or turn produces a small compact protrusion, which could fit into a depression or groove on the surface of a protein with which it interacts.

This work was supported by the US National Science Foundation and the National Institutes of Health. J.L.S. is a fellow of the Arthritis Foundation.

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Received November 13, 1975; accepted February 17, 1976.

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Systematic nomenclature for the RNA polymerase genes of prokaryotes

THE DNA-dependent RNA polymerase of *Escherichia coli* (EC 2.7.7.6) consists of at least four distinct polypeptide subunits; *α*, *β*, *β′* and *σ* (ref. 1). Other subunits have been reported (for example, *ω* (ref. 1) and *σ′* (ref. 2)), but their importance in transcription remains uncertain. Many prokaryotes are known to contain RNA polymerases of closely similar subunit composition, including a wide range of bacteria[3] and a blue-green alga[4]. Until recently, only the structural gene for *β* had been identified in *E. coli*[5]. It is located[6–10] at 88.5 minutes on the recalibrated genetic map[11], and is characterised principally by mutations conferring resistance to specific drugs. The *β*-gene has thus acquired several synonyms derived from the mutant phenotypes, including *rif* (for resistance to rifampicin)[8], *stv* (for streptovaricin)[9], and *stl* (for streptolydigin)[10,14]. More recently the structural gene for the *β′* subunit has been characterised in *E. coli*; it is adjacent to the *β* gene[13], and in the same operon[14–16]. Finally, the gene coding for *α* has been located at 72 minutes[11,17–19]. We now propose a systematic nomenclature for the RNA polymerase genes of *E. coli*, which could equally be applied to other prokaryotic organisms. We have consulted other workers in the field, whose comments have been assimilated into our proposal. The scheme, which is in line with the recommendations of Demerec *et al.*[20], is as follows.

(1) The symbol *rpo* should be applied to the structural genes coding for RNA polymerase subunits, and to their regulatory genes and sites. (The symbol *rna*, proposed in 1968 (ref. 7), has not come into general use[11].)

(2) The genes for the *α*, *β* and *β′* subunits should be called *rpoA*, *rpoB* and *rpoC*, respectively. We suggest that

Table 1 Revised names of some RNA polymerase mutations

New name	Former name	References
	Escherichia coli K12	
*rpoA*109	*gro*–109	18, 19, 21
*rpoB*1	uv1	16
*rpoB*2	A2	16
*rpoB*3	*rif*$^{\mathrm{d}}$	22
*rpoB*4	*rif*-r4	23
*rpoB*5	*rif*-r-5	24
*rpoB*6	*rif*-6 or *rif*$^{\mathrm{r}}_{\mathrm{RCB6}}$	25
*rpoB*7	7	16
*rpoB*8	K$_{\mathrm{M}}$7	26
*rpoB*9	XR9	16
*rpoB*10	*rif*-r$_{\mathrm{d}}$	27
*rpoB*11	*rif*-r-1	24
*rpoB*12	*rif*$^{\circ}_{\mathrm{amD12}}$	14, 16, 28
*rpoB*15	*rif*$^{\circ}_{\mathrm{amB15}}$	28, 29
*rpoB*17	XR17	16
*rpoB*18	*stl*-r-18	30, 31
*rpoB*19	*rif*-r-18	30
*rpoB*20	BT20	16
*rpoB*21	(*stl*$^{\mathrm{R}}$)	10
*rpoB*22	ts22	30, 31
*rpoB*25	*rif*-rB5	32
*rpoB*35	ST35	16
*rpoB*38	*rif*$^{\circ}_{\mathrm{amIII/8}}$	14, 16, 28
*rpoB*44	ST44	16
*rpoB*49	ST49	16
*rpoB*64	*rif*-r64 (SD)	33
*rpoB*65	ST65	16
*rpoB*70	*rif*-rJ7	28, 32
*rpoB*71	ST71	16
*rpoB*74	ST74	16
*rpoB*76	*rif*-r-76	34
*rpoB*80	ST80	16
*rpoB*90	ST90	16
*rpoB*91	ST91	16
*rpoB*93	J93	16
*rpoB*96	ST96	16
*rpoB*101	ST101	16
*rpoB*110	ST110	16
*rpoB*115	*rif*$^{\circ}$B115 amber	35
*rpoB*122	R122	16
*rpoB*123	R123	16
*rpoB*124	R124	16
*rpoB*130	R130	16
*rpoB*143	ts143	30, 31
*rpoB*165	ts65	30, 31
*rpoB*171	ts71	30, 31
*rpoB*184	D184	16
*rpoB*195	*stv*195	36
*rpoB*201	*stl*1	12
*rpoB*203	XH203	16
*rpoB*245	XH245	16
*rpoB*274	XH274	16
*rpoB*347	XH347	16
*rpoB*501	*rif*501	37
*rpoB*507	*rif*507	38
*rpoB*514	R514	16
Δ (*argBH-rpoB*) 18	Δ18	14, 16
*rpoC*1	tsX	39, 13
*rpoC*2	B2	16
*rpoC*3	cs1	23
*rpoC*4	4, ts-4	16, 40
*rpoC*17	XH17	16
*rpoC*25	XH25	16
*rpoC*38	B38	16
*rpoC*47	B47	16
*rpoC*55	ST55	16
*rpoC*56	XH56	16
*rpoC*87	ST87	16
*rpoC*110	XH110	16
*rpoC*120	R120	16
*rpoC*202	XH202	16
*rpoC*212	XH212	16
*rpoC*233	XH233	16
*rpoC*254	XH254	16
*rpoC*284	XH284	16
*rpoC*385	XH385	16
	Salmonella typhimurium LT2	
*rpoB*4	*rif*4	*
*rpoC*32	ts32	*
	Bacillus subtilis NCTC 3610	
*rpoB*3	*rfm*3Y	41

*Personal communication from A. Wright.

the gene for σ be *rpoD*, and that any further structural genes should continue the alphabetical sequence.

(3) The promoter for the *rpoB,C* operon[14] should be designated *rpoP*. We suggest that further regulatory genes and sites, controlling *rpo* structural genes, should continue the alphabet from *rpoO*.

(4). All *rpo* mutations should be identified by the gene letter, followed by a number. We strongly support the principle[20] that allele designations should not attempt to convey phenotypic information.

Table 1 lists some well-characterised mutations established in the literature, together with their agreed new names. We will gladly coordinate the future naming of mutations.

Table 1 lists some well-characterised mutations established *E.coli*[11].

We thank many colleagues for support and help in the shaping of this nomenclature, particularly the following: S J. Austin, B J. Bachmann, D. Boyd, R. R. Burgess, R. Calendar, I. V. Claeys, R. Doi, H. Echols, G. R. Hartmann, A. Heil, A. Ishihama, R. B. Khesin, J. B. Kirschbaum, J.-P. Lecocq, R. Losick, B. Low, H. Matzura, D. McConnell, J. H. Miller, B. Molholt, M. Nomura, M. Oeschger, P. Palm, L G. Silvestri, A. L. Sonenshein, W. Szybalski, A. L. Taylor, I. Tittawella, A. Travers, A. Wright, T. Yura and W. Zillig. We thank the MRC for project and programme grant support.

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Received November 27, 1975; accepted March 10, 1976.

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Enthalpy–entropy compensation of oxamate binding by homologous lactate dehydrogenases

STUDIES[1,2] of the amino acid sequence and crystal structure of M_4 lactate dehydrogenase (EC 1.1.1.27) indicate that all binding interactions are of the weak, non-covalent type (proceeding with only minor free-energy changes). Because critical regions of the substrate and coenzyme-binding site seem highly conserved[3], and because non-covalent interactions are highly, if differentially, sensitive to the physical environment[4], we wondered how organisms living in widely different temperatures and pressures stabilise enzyme–ligand interactions. We have therefore compared pyruvate binding site functions of lactate dehydrogenases obtained from organisms living in different temperatures and pressures. We used a placental mammal, the ox, with a cell temperature of 37 °C; a marsupial, the possum (*Trichosurus vulpecula*), with an average cell temperature of 36 °C (ref. 5), two monotremes, the platypus (*Ornithorhynchus anatinus*), with a cell temperature of about 31 °C (ref. 6), and the echidna (*Tachyglossus aculeatus*), with a body temperature of 23–32 °C (ref. 7); the goanna (*Varanus gouldi*), an Australian lizard with a cell temperature ranging from about 20 up to nearly 40 °C (ref. 8); the Pacific coast dogfish (*Squalus acanthias*), with an average body temperature of about 15 °C; an intertidal sculpin (*Oligocottus maculosus*), living in varying temperatures between about 5 and 15 °C, and an abyssal teleost fish, *Antimora rostrata*, with a highly constant body temperature of 2–3 °C. All these, except the dogfish and *Antimora*, can be viewed as species adapted to 1 atm; by contrast the abyssal species must be able to tolerate pressures of up to several hundred atmospheres, while the dogfish may encounter pressures from 1 to about 100 atm (N. J. Wilimovsky, personal communication). We found that adjustments in enthalpic and entropic contributions to lactate dehydrogenase binding allowed each homologue to bind ligand with similar avidity at its respective "normal" temperature and pressure.

M_4 lactate dehydrogenase was isolated from skeletal muscle of each species, and in all but the dogfish and sculpin, was purified to homogeneity (thrice crystallised) by standard purification procedures as before[9]. Dogfish and sculpin enzymes were purified by affinity chromatography, using oxamate covalently linked to Sepharose B as described elsewhere[10]. Enzyme activity was assayed by following the change in A_{340} due to NADH oxidation in a Unicam SP 1800 recording spectrophotometer. Cuvette temperatures were kept constant (±0.1 °C) with a Lauda constant temperature bath and circulator. To study the effects of pressure, we used a high pressure cell of 1-cm light path, with sapphire windows, installed into a Unicam SP 1800 spectrophotometer, as previously described[11].

An indication of how the substrate-binding site is adjusted with respect to the environment can be obtained from studies of oxamate inhibition of the enzyme. Oxamate, isoelectronic and isosteric with pyruvate, is considered a perfect structural analogue of the substrate[2]. During oxamate or pyruvate binding, a negative charge must be neutralised, and the overall process should be favoured by low temperature and low pressure[4]. If that were simply the case, then the lower the temperature, the higher the enzyme affinity for oxamate, and the enzyme from a low temperature organism (such as *Antimora*) would be expected to have a much higher affinity at its biological temperature than would a homologue in a high temperature organism. This situation was not observed. Although the overall effect of temperature on the K_i for oxamate is qualitatively similar, the position of each curve on the temperature axis

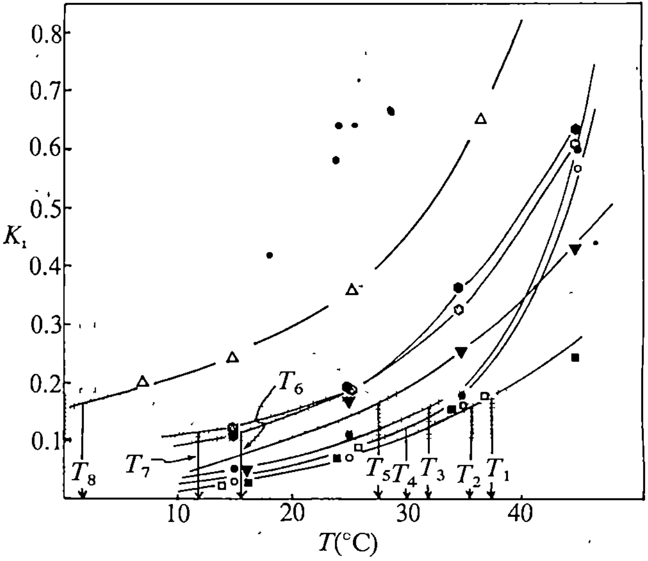

Fig. 1 A plot of the K_i for oxamate against temperature in °C for M_4 lactate dehydrogenases from eight vertebrate species. Arrows from each curve refer to the approximate body temperature in each case. T_1 refers to the ox; T_2, the possum; T_3, the platypus; T_4, the goanna; T_5, the echidna; T_6, the dogfish; T_7, the sculpin; T_8, *Antimora*. The K_i for oxamate was determined at *p*H 7.5, 100 mM Tris-HCl buffer, 25 °C, at minimally two concentrations of pyruvate, between 0.25 mM and 1 mM, and at 0.1 mM NADH. Total volume was 1 ml. Pressure was constant at 1 atm. K_i values (mM) are accurate to within ±10%. Each K_i value represents an average of three determinations from Dixon plots of 1/velocity against oxamate concentration.

Table 1 Thermodynamic parameters for association of oxamate and binary lactate dehydrogenase–NADH complexes for M_4 forms of the enzyme from species adapted to different temperatures and pressures

Enzyme source	Average biological temperature (°C)	ΔG^0 (kcalorie mol^{-1})	ΔH^0 (kcalorie mol^{-1})	ΔS^0 (calorie mol^{-1} K^{-1})	ΔV^0 (ml mol^{-1})
Ox	37	−5.7	−15.4	−32.3	36
Possum	36	−5.7	−15.2	−31.9	Unknown
Platypus	31	−5.4	−14.5	−30.5	Unknown
Goanna	20–40	−5.6	−14.5	−29.9	Unknown
Echidna	23–32	−5.2	−14.0	−29.5	Unknown
Dogfish	15	−5.1	−11.0	−19.8	5.4
Sculpin	5–15	−5.1	−8.2	−11.1	10.0
Antimora	2	−4.7	−6.1	−4.9	0

K_i values from which these data are calculated are reproducible to within ±10%. Values for ΔG^0, ΔS^0 and ΔV are calculated for data obtained at 298 K.

roughly correlates with the temperature in which each enzyme homologue normally functions (Fig. 1). In each case the affinity of the binary complex for oxamate is similar at each biological temperature (although Fig. 1 shows that at any given temperature enzymes adapted to high temperatures bind oxamate more tightly than do their homologues in organisms adapted to the cold).

Similar observations have been made for the apparent Michaelis constant for pyruvate[12]. These and our own studies imply some selective advantage if, in each species, these enzymes have a particular range of substrate affinity. Atkinson has argued persuasively that an enzyme's affinity for its substrates is designed by natural selection for optimal function within the *in vivo* concentration range through which the substrates fluctuate[13]; since pyruvate concentrations are likely to be similar in muscles of different vertebrates, similar binding ability at each respective biological temperature should perhaps be expected. Be that as it may, the question remains as to how such fine thermal adaptation of lactate dehydrogenase-binding function is achieved, particularly with a ligand as apparently simple as oxamate.

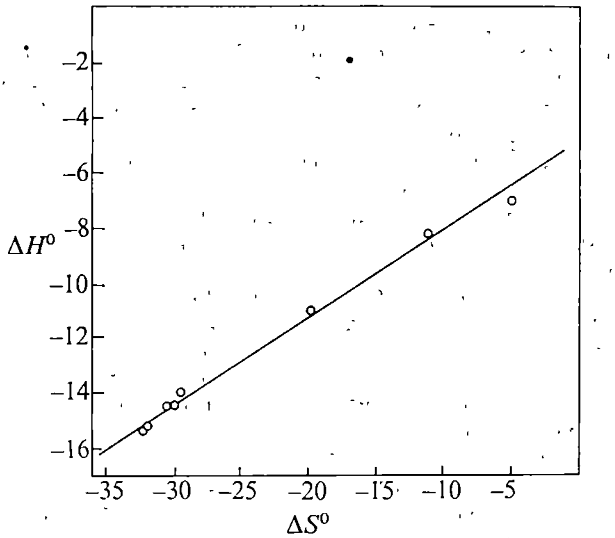

Fig. 2 A plot of $\triangle H^0$ against $\triangle S^0$ associated with oxamate-binding by the binary NADH–enzyme complex for lactate dehydrogenases isolated from vertebrate species adapted to different physical environments. The enthalpy change is in calorie mol^{-1} while the entropy change is in calorie mol^{-1} K^{-1}. The data for this plot are calculated from K_i values that are reproducible to within ±10%

One possible mechanism would involve structural adjustments at the substrate binding site *per se* so as to 'optimise' binding at different biological temperatures. Although this may be the simplest hypothesis to fit the data, current evidence indicates that critical regions of the enzyme-binding sites are highly conserved[1–3]. Therefore, we tentatively reject the hypothesis.

A second basis for this rejection perhaps is to be found in a careful analysis of the thermodynamic parameters for association of oxamate and the binary complex. Table 1 shows that the lower the biological temperature of normal function, the lower the enthalpy and entropy changes occurring during oxamate binding. Although the differences between some of the enzyme homologues are small, at the extremes of the spectrum obtained the differences are impressive: the values for ΔH^0 for oxamate binding by the possum or ox homologues are nearly 1.5 times the ΔH^0 for the dogfish enzyme and are 2.5 times the ΔH^0 for the *Antimora* M_4 enzyme. The quantitative relationship between ΔH^0 and ΔS^0 for oxamate binding by the different lactate dehydrogenases (Fig. 2) seems to be an example of "enthalpy–entropy compensation". The slope of the plot in Fig. 2 corresponds to a "compensation temperature" of about 325 K, and is similar to that obtained for ADP binding by glutamate dehydrogenase[14] and for activation parameters for vertebrate lactate dehydrogenases[15,16]. The compensation temperature range, 250–325 K, is observed so frequently that some workers believe the phenomenon to be a consequence of the properties of water as a solvent[17,18]. In general, the more water that must be removed from the substrate or from the enzyme, the higher the enthalpy and entropy changes during formation of an enzyme–substrate complex. That some such effects contribute to the thermodynamics of coenzyme and substrate binding by lactate dehydrogenases is supported by several observations. First, in the ternary complex, the position of a collapsed loop excludes bulk water from the active site, while in the apoenzyme it extends well into the solvent[1]. In this sense at least water must be removed from the enzyme during stabilisation of the ternary complex. Second, water must also be removed from the coenzyme during binding because both negative charges on the pyrophosphate are solvated in solution, but only one is solvated in the ternary complex[1,2]. Third, the degree of surface hydration of the apoenzyme compared with the ternary complex almost certainly differs significantly, although structural studies of lactate dehydrogenases have not quantified this characteristic. Fourth, enzyme–water interactions seem to vary between species, with homologues from cold-adapted species tending to have notably oily surfaces, a characteristic observed for both pyruvate kinases and glyceraldehyde-3-phosphate dehydrogenase (G. N. Somero, personal communication). Thus there seems ample possibility to adjust the thermal dependence of enzyme–ligand binding by adjusting the energetic contributions of solvation effects anywhere in the enzyme structure.

If that were true in our case, volume changes associated with oxamate-binding by lactate dehydrogenase homologues should also vary in a regular manner, the larger the ΔH^0 and ΔS^0, the larger the expected volume change (ΔV) (ref. 17). At least in a qualitative sense, this prediction is realised in preliminary studies of total volume changes during oxamate binding. Thus the expected volume increase, associated with charge neutralisation[4], appears with M_4 homologues from high cell temperature organisms[19]. In contrast, oxamate binding by the piscian binary complexes proceeds with a greatly reduced volume change (Table 1); with *Antimora* enzyme the ΔV is so close to zero it cannot be measured accurately by the techniques used. These studies are in progress and will be published in detail elsewhere. In these cases the simplest interpretation is that the positive ΔV occurring on charge neutralisation of oxamate is at least partially compensated (or accommodated) by a negative volume change of similar magnitude, but mediated by changes in the degree to which water can orient around water-density-modifying groups such as carboxylates, peptide linkages, or non-polar amino acid residues. In consequence, the total ΔV is lower than in the ox homologue. As with activation parameters[20], nothing need be assumed about where in the enzyme structure these events occur, although they probably occur somewhere removed from critical interacting regions of the substrate-binding site *per se*.

This work was supported by a grant (P.W.H.) from the National Research Council of Canada. We thank Dr Paul Dreizen for the *Antimora*.

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Possible Gibbs–Donnan mechanism for cation uptake

MANY freshwater animals take up metal ions from solution in the surrounding water, often by active transport against a concentration gradient. The uptake of ions would be helped, and their loss opposed, by any means which increases the electrochemical potential of the ions in question in the external medium. One such means would be for the animal to secrete into the water a cationic substance to which the skin is not permeable; this would set up a Gibbs–Donnan effect (whereby an unequal distribution of diffusible ions is established at equilibrium on the two sides of a membrane if one side contains a non-diffusible ion) on the ions which are to be absorbed. The presence of such a mechanism in nature would be evident from the accumulation in the growth medium of cationic substances whose effect on the uptake of metals could be demonstrated experimentally.

For instance, the pulmonate snail *Biomphalaria glabrata* was shown by Thomas *et al.*[1,2] to secrete a substance which might act this way. The growth of the snails in these experiments was on the whole limited by the amount of calcium which could be absorbed, but the snails' secretion increased their growth rate and lowered the minimum concentration from which net uptake of calcium could be achieved. When the substance was allowed to accumulate in a limited volume of water (< 200 ml) for 3 d, the calcium concentration was reduced by the snails' uptake to ~ 2.5×10^{-5} M. But when the secretion was prevented from accumulating by continuous replacement of the medium, net uptake was not possible from solutions $\lesssim 2.5\times10^{-4}$ M. Addition of the partially purified secretion of other snails to the continuously replenished medium lowered the limiting calcium concentration from which net uptake was possible.

This model implies that the secretion of *B. glabrata* could increase the electrochemical potential of calcium tenfold. It is possible to calculate the effective concentration of non-permeant cations required to produce this effect, assuming that no other cations are present in significant amount. We can treat the system as though Ca^{2+} were partitioned at equilibrium between two compartments separated by a membrane permeable only to Ca^{2+} and to a common anion such as HCO_3^-. The molar calcium concentration in the compartment from which the non-permeant cation is absent (representing the interior of the snail) would then be $m'_{Ca} = 2.5\times10^{-4}$ and in the compartment where the non-permeant cation is present (representing the exterior medium) the calcium concentration is $m_{Ca} = 2.5\times10^{-5}$. If the substance in question has molecular charge z, the molar concentration, m_p, required will be given by the Gibbs–Donnan equation

$$\gamma_\pm{}^3 m_{Ca}(2m_{Ca}+zm_p)^2 = 4\gamma_\pm'^3 m'_{Ca}{}^3$$

where $\gamma_\pm$, $\gamma_\pm'$ are the activity coefficients of the calcium salt in the compartments with and without the non-permeant cation, respectively. Substituting the calcium concentrations given above, and assuming the activity coefficients equal, we obtain the value $zm_p = 1.6\times10^{-3}$, which seems reasonable.

The Gibbs–Donnan effect will operate on all permeant cations, to an extent depending on their relative concentrations and their valence. (There is more effect on multivalent than on univalent ions.) The presence of an excessive concentration of another permeant ion such as Na^+ would reduce the enhancement of the electrochemical potential of Ca^{2+}. Other things being equal, the uptake of permeant cations would be favoured in approximate proportion to their external concentrations. Other non-permeant ions would enhance or reduce the Gibbs–Donnan effect depending on their concentrations inside and outside the animal and on their net molecular charges.

Apart from the identification of cationic macromolecular substances in the secretions of freshwater animals, this model could be confirmed by reproducing the Gibbs–Donnan effect with cationic polymers such as diethylaminoethyl dextran or polylysine. The presence of these in the water at quite modest concentrations ought to assist the uptake of calcium and stimulate the growth of animals as does the substance studied by Thomas.

It is not to be expected that such a mechanism would be useful to all freshwater animals, but only to those living in limited volumes of still water in which the hypothetical substances could accumulate. Because the mechanism would act equally well to retard loss as to promote uptake of ions, it would be of greater value to animals vulnerable to such loss; for example, it would be more likely to occur among gastropods, which are liable to dissolution of the shell, than among crustaceans, whose calcareous structures are protected by an impermeable cuticle.

I am indebted to Dr A. G. Ogston for his suggestions and criticisms.

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Received January 26; accepted February 26, 1976.

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matters arising

Evidence for two sodium sites on the external aspect of Na–K pump in human erythrocytes

THE Na–K pump, which actively transports Na outward and K inward, is inhibited by external Na in human erythrocytes. This inhibition has been interpreted as being either "dead end" at one of the two putative external K sites[1], or as allosteric, occurring at a third external site on the pump[2]. Both of these models assume that occupation of the pump by a single external sodium ion is sufficient to inhibit the pump. In the "dead end" model, occupation of one of the K pump sites by Na inhibits pump turnover[1]; in the allosteric model, occupation of the external sodium site decreases the affinity of the K pump sites for K, but does not inhibit K translocation once the K sites have been occupied.

The experiments which led to these models have been done at relatively low external K concentrations, [K]$_0$, well below 1.5 mM, the [K]$_0$ at half-maximal pump flux in high Na solutions[3]. When inhibition by Na of ouabain-sensitive K influx, $^1M_K{}^P$, was determined in solutions containing 2 mM [K]$_0$, results such as those in Fig. 1 were obtained. The results, presented as a Dixon plot[7] ($1/{}^1M_K{}^P$ against [Na]$_0$), fit a parabola, suggesting that two external sites must be occupied for Na to inhibit ouabain-sensitive K influx at the [K]$_0$ used. These results contrast with those obtained by Cavieres and Ellory[2] at lower [K]$_0$. Dixon plots of their data gave straight lines for K concentrations between 0.1 and 0.35 mM, and hyperbolae for concentrations less than 0.1 mM. The difference in the results at 2 mM [K]$_0$ compared with lower [K]$_0$s might be considered in terms of the relative affinities of the two sodium sites depending on whether one K ion or two occupy the external aspect of the pump. The form XK may have such a high affinity for Na at the first Na site that the effect of occupation of this site is not seen at the lower [K]$_0$s. This would be analogous to measuring K activation of the pump in Na-free solutions where the curve appears hyperbolic unless extremely low [K]$_0$s are used, where a slight sigmoidicity may become apparent. In this scheme the form KXK would have a lower affinity for Na than XK and the occupation of both Na sites would be demonstrable kinetically at a higher [K]$_0$.

Another useful way of examining extracellular Na sites on the pump is to look at the effects of [Na]$_0$ on the rate of ouabain binding. External Na increases the rate of ouabain binding to the pump, whereas external K inhibits it. The effect of Na has been attributed both to a direct action, independent of K[8], and to an action limited to restricting access of K^+ ions from the sites at which they inhibit ouabain binding[1]. In the experiment shown in Fig. 2, we altered the rate of ouabain binding (shown as the rate constant of binding), but not the K pump rate, by increasing [Na]$_0$ in a solution in which [K]$_0$ was sufficient to saturate the pump at all Na concentrations used. Thus, increasing [Na]$_0$ caused no inhibition of $^1M_K{}^P$, but the rate of ouabain binding increased fourfold. This shows that Na may occupy at least one external site without inhibiting the pump, and supports a configuration of the external aspect of the pump, $_KX_K{}^{Na}$, suggested by Cavieres and Ellory[2]. Figure 1, however, demonstrates that at least two external sites must be occupied by Na to obtain inhibition at 2 mM [K]$_0$. In low [K]$_0$ and saturating [Na]$_0$, one would expect

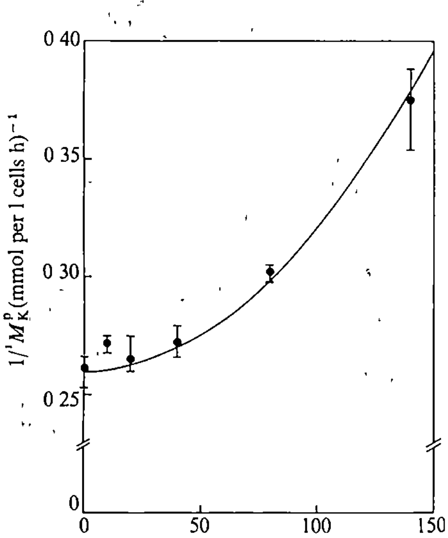

Fig. 1 Inhibition of ouabain-sensitive K influx by external Na in human red cells. Cellular cation concentrations were altered by reversibly increasing cation permeability using nystatin[4], so that [Na]$_i$ = 88 mmol per l cells and [K]$_i$ = 8 mmol per l. Unidirectional K influxes were measured using ^{42}K as before[5] in a solution containing (mM): KCl, 2; choline Cl+NaCl, 148; glucose, 5; Tris-HCl, 10 (*p*H 7.5). The cells were exposed to ^{42}K for 30 min. The maximum difference in [K]$_i$s in aliquots of cells at the various [Na]$_0$s, measured after incubation in ^{42}K, was 0.9 mmol l^{-1}. The resultant differences in $^1M_K{}^P$ due to stimulation by [K]$_i$ would be of the order of 5% (ref. 6). Ouabain-sensitive fluxes were determined after preincubation of cells in 10^{-3} M ouabain for 10 min at 37 °C in K-free media. Results are plotted as the reciprocal of the pump influx, $1/{}^1M_K{}^P$, against external Na concentration, [Na]$_0$. The curve was drawn from the equation, $1/{}^1M_K{}^P = 0.26+(6.0\times10^{-6})\,[\mathrm{Na}]_0{}^2$. The points represent means of triplicate determinations. The maximum range of values about a mean was 3.9% of the mean, and the average range was 2.4%. Similar results were obtained in another comparable experiment.

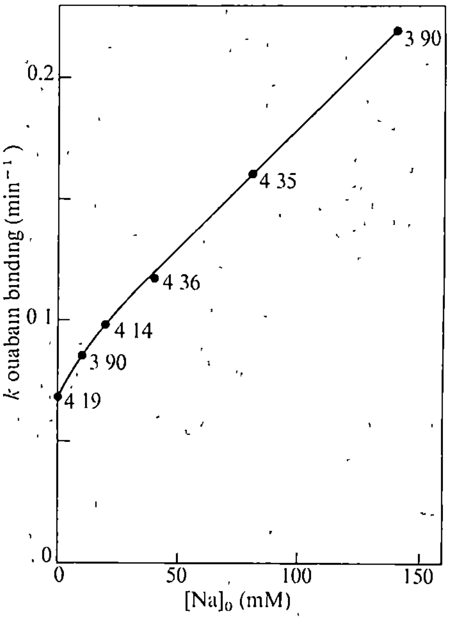

Fig. 2 Promotion of the rate of ouabain binding by external sodium. Cellular cation concentrations were altered using nystatin as described in Fig. 1 so that [Na]$_i$ = 53 mmol per l cells and [K]$_i$ = 43 mmol per l. The rate of ouabain binding was determined by measuring the onset of inhibition of the pump after preincubation for various times in solutions containing 10^{-6} M ouabain, 11 mM KCl, 5 mM glucose, 10 mM Tris-HCl (*p*H 7.5) and NaCl as indicated, with NaCl+choline Cl = 140 mM. Ouabain binding was stopped by addition of ice-cold isotonic choline Cl. After washing by centrifugation, unidirectional influx of K was measured[5] in a medium containing 11 mM KCl, 140 mM choline Cl and Tris-HCl and glucose as before. Thus for each Na concentration a time course of ouabain binding was measured, and the rate constant, *k* (min^{-1}), of binding was determined from the slope of the curve for the time course. The numbers on the figure are ouabain-sensitive K influxes (in mmol per l cells × h) measured in 11 mM [K]$_0$ and Na concentrations indicated but in the absence of ouabain. Fluxes were determined in triplicate and ouabain binding rates in quadruplicate.

the two sites to be filled by Na. Given the "limiting non-zero ouabain-sensitive K influx" described by Cavieres and Ellory in these conditions[2], the minimum requirement of a model for the pump is that two sites can be occupied by Na and one by K simultaneously. Two possibilities are that (1) the external pump form $^{Na}X_K{}^{Na}$ is capable of active K translocation, or (2) additional K or Na sites (for example, the form $^{Na}{}_KX_K{}^{Na}$) are present on the external aspect of the pump.

This work was supported by a grant from the National Institutes of Health.

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CAVIERES AND ELLORY REPLY—The finding of an upwards deflection of the $1/v$ against $[Na]_0$ curve would certainly be very interesting. Unfortunately the deviation from a straight line in the data presented[1] is effectively based on only one point ($[Na]_0 = 140$ mM), where the flux is 13.6% lower than it would be for a straight line fit. From the legend to Fig. 1 it is apparent that cell K (~ 8 mM) can be ~ 10% lower in the cells incubated in 140 mM Na medium, thus reducing the K influx by ~ 5% (ref. 6). If the further contributions of experimental error (up to 3.9%) and the scatter (for example, for $[Na]_0 = 10$ mM) are considered, it becomes difficult to defend the departure from the straight line fitted through the remaining data. In fact, from inspection of our earlier experiments carried out at a $[K]_0$ of 1 mM, we could find no evidence for deviation from a linear relationship. A final possibility (although unlikely), is that the difference in internal K between our experiments (K ~ 50 mM) and theirs (K ~ 8 mM) may have a role.

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Haze in the stratosphere from the Fuego eruption

SCORER[1] reported seeing an extended haze layer during Concorde flights at heights up to 17 km (56,000 feet) on September 1, 1975 from London to Gander and back. He assumed that this was the result of large scale ascent of normal tropospheric dust. On the premise that this was indicative of the normal interchange mechanisms across the tropopause, he then concluded that the theories which have led to the recent 'ozone depletion scares' are based on 'nonsense' suppositions regarding the nature of the vertical transport in the stratosphere.

A much more likely explanation of the haze layer observed by Scorer is that this was aerosol remaining from the material injected into the lower stratosphere by the violent eruptions in mid-October 1974 of Volcan de Fuego in Guatemala. A number of different investigators reported the appearance of a haze layer (or layers) between ~16 and 20 km during the following months[2]. Further observations during the past year, with lidar[3], twilight measurements[4], dust sonde[5], and searchlight measurements[6] indicate the continued presence of this dust layer over Hawaii, Puerto Rico, and northern midlatitudes. It is probably still present over the Arctic, but it is not known whether any dust reached the Southern Hemisphere. According to the lidar data the layer has diminished slowly since early spring 1975 with a residence time of ~6 months—about half that of large volcanic events. Measurements at several locations show, however, that purple twilight glows are now (after a minimum in April) nearly as strong as those of winter 1974–75. The haze phenomenon described by Scorer has often been studied in colour photographs obtained by time lapse cameras flown on balloons, mostly over New Mexico[7]. In the flights from December 1974 to the most recent in October 1975, strong haziness near the solar azimuth extends to ~20 km. If the Sun is not high up, a rather sharply bottomed haze band appears at a balloon altitude of ~16 km. When the balloon is in the main layer, the distant horizon disappears, and thin layers may be noticed briefly. When the balloon is leaving the layer the distant haze top becomes sharp and edged by deep blue sky. Optical measurements showed very little aerosol in the region of the layer in question during 1972–74, but some thin layers could always be detected in the balloon photographs because of the great path length.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Zenith attenuation of solar radiation by the Fuego dust was hardly >1%. Therefore the change of Earth temperature was ~0.2 K only[8], and the effect on vertical transport less than that suggested by Scorer.

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SCORER REPLIES—A characteristic of the occasion on which I observed the haze was that there were cirrus clouds above the tropopause and the tropopause itself was not apparent to the eye. After a year I would have expected purple twilight to have been much decreased, as the note says it was in April, and the large vertical extent of the haze suggested that it was not very old, otherwise it would have been sheared over into thin layers, of which several would be unlikely to be found together over one geographical location.

On the assumption that such layers come from volcanic eruptions, anyone determined enough can, of course, ascribe them to the one they think to be the most likely cause; but it is important to recognise that the assumption can dominate thinking about these phenomena. That explanation is not "more likely" except after acceptance of the assumption.

I do not see what attenuation of solar radiation or change of Earth temperature have to do with the radiative equilibrium, which I supposed was dominated by a change in the absorption of the outgoing long waves.

Reiter[1] gives a spread of residence times for stratospheric air ranging from 80 to 1,000 d at 20 km, which seems reasonable. Therefore to ascribe finality to any explanation of the haze is possible only if it can be traced accurately back to its origin. What I am suggesting is that there are probably many manifestations of the mass-interchange mechanism not yet properly investigated, and that hurricanes could be involved to an important extent.

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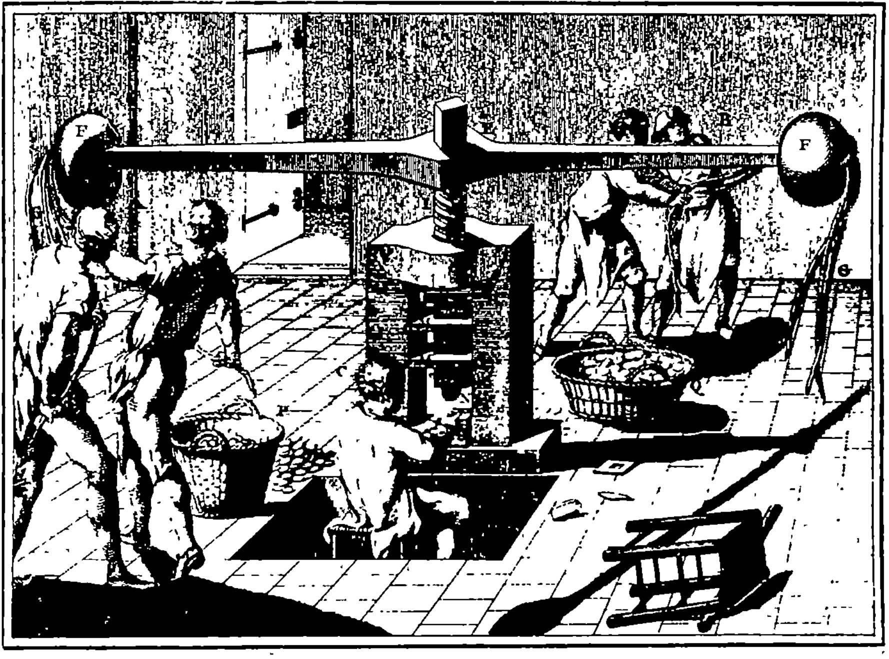
reviews

A double life

J. W. Herivel



Machinery of the Mint, as illustrated in the Encyclopédie *(Paris, 1771). The great screw-press (a fly-press in modern terms) which impressed the round blank.*

THE period covered by this volume witnesses the continuation, and exacerbation, of the controversy between Newton and Flamsteed, and the initiation of that with Leibniz over the question of priority in the calculus. Throughout the period Newton continues in his dual role as Master of the Mint and President of the Royal Society. His activities in the former role are copiously documented, those in the latter much less so. Compared with the previous period, and excluding the letters and papers relating to the Mint, there is perhaps a certain narrowing of Newton's correspondence in this volume. Thus there are now no letters to or from Locke or Halley. But any such restriction is more than compensated by the presence of the whole of the magnificent correspondence with Cotes over the preparation of the second edition of the *Principia*. Although Newton had long ceased to be mathematically or scientifically creative, the correspondence with Cotes proves that his intellectual energies remained undimmed. Apart from certain of the Mint letters, he also continues to write in a style marked by that concision, clarity, and strong sense of word order, first displayed in his 1672 paper on colours.

Almost all the Mint letters and memoranda are here published for the first time. On the whole, they are not the most interesting of Newton's letters, but there are exceptions, such as the memorandum (816a) "Of the assaying of Gold and Silver, and the making of indented Triall-pieces and trying the moneys in the Pix"; a measure of the importance which Newton himself attached to this memorandum is given by the fact that it exists in no fewer than eight drafts among the Mint papers. Among the letters relating to the Flamsteed controversy, a blistering letter (825) to Flamsteed about which Newton probably had second thoughts, and a hitherto unpublished letter (853) to Flamsteed from his "quondam friend and not yet profligate Enemy" Halley are noteworthy. Also another hitherto unpublished letter (978) to Flamsteed from a Committee of the Royal Society requiring the unfortunate Astronomer Royal to send them a copy of his observations for 1712 in accordance with "Her Majesty's Most Gracious Letter of 12 December 1710"—the warrant (reproduced at 814) extracted by Newton from the Government, requiring Flamsteed to surrender up his observations for any given year within six months of its ending.

Surprisingly few letters and memoranda relating to the Newton–Leibniz controversy are found in this volume But as the editors point out in their introduction, this is no index of the time which Newton actually devoted to the controversy, inevitably at the expense of other activities including his correspondence with Cotes over the second edition of the *Principia*. When drawing up his celebrated edition of this correspondence, Edleston did not have access to the Portsmouth Collection. This accounts for the appearance of no less than 12 new letters between Newton and Cotes here published for the first time. Taken together, these represent such a substantial addition to the Newton–Cotes correspondence as almost to call for a new edition, preferably with a fairly detailed exposition of Newton's lunar theory in an appendix For although the introduction and notes to the present volume are very helpful in this respect, they still leave much obscurity occasioned in part by Newton's habit of carrying out long lines of reasoning without the use of symbols.

Among the new letters, number 829 throws interesting light on Newton's original intention to thank Cotes publicly for his vast (unpaid) assistance with the preparation of the second edition of the *Principia*. As suggested by D. T Whiteside, the apparent cooling in Newton's relations with Cotes towards the end of the enterprise may possibly have been due to Cotes's failure to notice the error in Proposition 10 of Book II first drawn to Newton's attention by Johann [I] Bernouilli, (see 951a) in circumstances which the controversy with Leibniz rendered embarrassing to Newton. Of letters and memoranda on other miscellaneous topics the following, all hitherto unpublished, are noteworthy; number 802, in Newton's most pithy

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The Correspondence of Isaac Newton. Vol. 5: 1709–1713. Edited by Rupert A. Hall and Laura Tilling. Pp. li+439. (Cambridge University: London, October 1975. Published for the Royal Society.) £20.

Mr Herivel is a reader in the Department of the History and Philosophy of Science at Queen's University, Belfast, Northern Ireland.

style, with its triumphant announcement of his purchase of new premises for the Royal Society; number 878, in which Newton announces the death of Colonel Robert Barton to his widow's father leaving the latter "discretion to let her know it by such degrees and in such manner as may least afflict her"; number 918, of interest for Newton's views on gravitational attraction; and number 961a, an important memorandum *De Vi Electrica* concerned with Newton's views on attractive forces. The volume is brought to a fitting close by Bentley's letter (1002) to Newton, announcing the publication of the second edition of the *Principia*.

No review of this volume would be complete without a tribute to the late J. F. Scott, editor of volume 4, whose untimely death, following so closely on that of H. W. Turnbull, was such a loss to this great project. It is to be hoped that the present editors will be able to carry it through to completion. Their editing of the present volume, including the footnotes and an admirable introduction, maintains the high standards set by preceding volumes. and makes it clear that no better choice of editors could be made for the project's final stages □

Universal pictures

The Amazing Universe. By Herbert Friedman. Pp. 200. (National Geographic Society: Washington, DC, 1975.) $4.25.

THE May 1974 issue of *National Geographic* included a feature on "The Incredible Universe"; this book develops the theme further with a new text by Herbert Friedman and a lavish use of colour illustrations. I am slightly disappointed, however, that the most has not been made of the opportunity to provide an account of the most intriguing theories of the new astronomy, and that the book largely rests on its illustrative laurels. Nevertheless, it is remarkably good value and helps to plug the gap between conventional 'popular' astronomy books and the revolutionary developments that have taken place in the past ten years or so.

Friedman has concentrated on discussion of the techniques of the new astronomy, which have opened up new parts of the spectrum and other areas to observation, but he does not complete the guided tour of theoretical work we have been led to expect. On solar neutrinos, for example, after describing the Davis experiment, we are merely told that "if the cleaning-fluid trap produces no neutrinos, the mystery . . . may force solar physicists to re-examine their theories"; the "mystery" of Sco X-1 is described as "a continuing challenge for today's Eddingtons", when in fact the source now seems to be well understood; Weber's gravitational radiation claims are to be "interpreted with extreme caution", when there is now a clear consensus which the author does not report; and the mention of apparent faster-than-light separations in some QSOs is followed by the vague comment that "some ingenious explanations for this have been put forward", when those explanations seem both straightforward and a clear indication of the illusory nature of the measured superlight speeds.

Even the professional astronomer may enjoy the pretty pictorial record, and as far as it goes *The Amazing Universe* is good value both in appearance and scientific content. It would make an ideal gift for a young reader freshly interested in astronomy, with the proviso that the donor should take care to follow this up with more meaty texts if the bait is taken.

John Gribbin

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Photochemistry

Concepts of Inorganic Photochemistry. Edited by Arthur W. Adamson and Paul D. Fleischauer. Pp. ix+439. (Wiley Interscience: New York and London, August 1975.) £13.30.

THIS volume gives an account of the developments in coordination photochemistry, each chapter being contributed by an authority in the field. Two chapters deal with photophysical processes: the first is a brief survey of the electronic spectroscopy of complexes, the second an account of the kinetics of photophysical processes based on concepts well established in organic photochemistry. Against this background are set chapters on charge-transfer photochemistry, substitutional photochemistry of first-row transition elements, and on the photochemistry of the heavier elements, of carbonyl complexes, of 1,3-diketonate chelates and of simple inorganic ions in solution. In each of these chapters the existing experimental information on selected systems is critically reviewed and an attempt is made to interpret the data in terms of principles governing the reactivity of the excited state. The last two chapters are more speculative. The first deals with photochemistry in the solid state, a challenging field of great importance in which the difficulties of obtaining quantitative information and of interpreting it, are formidable. The brief final chapter on photochromism and chemiluminescence concentrates on general principles. It includes the novel suggestion that the "thermally equilibrated excited state" in photochemical reactions may be identified with a particular modification of the "transition state" postulated in thermal reactions.

These are stimulating reviews which give an excellent account of the present state of a rapidly developing subject. Overlap between chapters has been kept to a minimum; a degree of uniformity in terminology and treatment helps to emphasise the common factors in different areas of the subject. A minor source of irritation, however, is the inconsistency in the use of units—the variety of captions for wavelength and wave number, for example.

The book will be welcomed by investigators in the wide field of inorganic chemistry. With its emphasis on concepts and interpretation it should be of general interest to many inorganic chemists.

M. I. Christie

Coherent calculus of subjective probability

Theory of Probability: A Critical Introductory Treatment, Vol. 2. By Bruno de Finetti Translated by Antonio Machi and Adrian Smith Pp. xviii+ 375. (Wiley: London and New York, 1975.) £10.50.

THIS is the second half of the English translation of Professor de Finetti's Italian *Theory of Probability*, and completes a work which, in the opinion of the foreword, "is destined ultimately to be recognised as one of the great books of the world" In reviewing the first half, besides sketching the background to the controversies which occupy the minds of many statisticians, I noted that it was the second volume which would carry Professor de Finetti's critical discussion of inductive reasoning and statistical inference, and that I awaited it with impatience (*Nature,* **251**, 262, 1974). Now that it has arrived, it is slightly disappointing, although in other respects the author presents an invigorating and wholly delightful account of some fascinating topics in probability theory. In treating these (chapters 7–10) he greatly strengthens his Bayesian position by his deft treatment of paradoxical examples (it would be too much to call these teasers of probability theory *paradoxes*). Studded with illuminating asides and revealing anecdotes, this part of the book is a delight. How many statisticians know that the points of inflexion of the normal distribution are at the one-standard-deviation points? Professor de Finetti knows that the tangents at these points cut the axis at the two-standard-deviation points, and such asides give the book its flavour.

Chapter 11, 'Inductive Reasoning; Statistical Inference', is the kernel of the book. "It is particularly instructive to consider the process by which new scientific theories are formulated. The first step is an intuitive one, arising out of some particular set of observations, but then various modifications are made as a result of more up-to-date results which suggest that this or that alternative theory provides a better explanation". So far so good, but what is to be the currency of explanation? I would say *likelihood,* but for de Finetti: "In essence, it is always a question of analysing the current state of information by means of Bayes's theorem". Later, "In our formulation, the problem of induction is, in fact, no longer a problem: we have, in effect, solved it without mentioning it explicitly. Everything reduces to the notion of conditional probability".

The author presumes on our acquiescence, and we look in vain for arguments which will lead the doubting majority to join the congregation of the Reverend Thomas Bayes: why should scientific hypotheses be treated like statistical events? If they should be, *then* the Bayesian position surely follows. for I believe de Finetti carries that part of his case—that the right calculus for events of uncertain outcome is the coherent calculus of subjective probability: it is, perhaps, his major contribution. Bayes, I think, would have approved; indeed, de Finetti could with profit have included an analyis of Bayes' paper in his book, for the one presages much that is in the other.

In fact, Bayes did not include scientific hypotheses in his scheme: that proposal is neo-Bayesian. But whatever we call it, it is the crucial issue. For me it is neither a reflection of how science does progress nor a paradigm for how it should. If universally applied it would reduce creative scientific activity to the level of accountancy. Coherence has its place, just like the double-entry book-keeping introduced by de Finetti's compatriots—the Lombard bankers of long ago: *c'est magnifique, mais ce n'est pas la guerre.*

But let each reader decide for himself. de Finetti's two volumes are essential reading. **A. W. F. Edwards**

Bowl (diameter 30 cm) found as part of a grave lot. New Mogollon pottery style of the 10th century AD, Mimbres area of south-western New Mexico. Decorated with a black-on-white design of enigmatic human figures, possibly representing the contrast between life and death or male and female. The hole puncturing the base, 'killing' the object, helped release the vessel's spirit into the next world. Taken from *The American Indians: Their Archaeology and Prehistory.* By Dean Snow. Photographs by Werner Forman. Pp 265. (Thames and Hudson: London, April, 1976.) £6.50.

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Circle No. 11 on Reader Enquiry Form.

The Blood of Sheep: Composition and Function. Edited by M. H. Blunt. Pp. xiii+224. (Springer: Berlin and New York, 1975.) DM 72; $31.

This is not a haematological text: the leukocytes receive scant attention, and the platelets and coagulation do worse. Instead it is a collection of chapters by different authors on body fluids, cells, carbohydrate and lipid metabolism, minerals, hormones, genetic markers, haemoglobins, immunoglobulins and anaemias. Each chapter is a factual review with little discussion, and occasional overlap with its fellows. More discussion would have been welcome and more selective editing could perhaps have provided the space. There seems little need in a work of this kind for a description of the actions of each hormone, irrespective of what is known about its presence in the sheep. The chapters vary between crisp, direct succinctness and a lengthy struggle to be comprehensive, and each provides numerous useful references. Nevertheless, because of its layout and index the book proves difficult to use as a reference work. Such criticism should not detract from its usefulness in bringing together a great deal of very interesting material for those centres where the sheep is a major object of study, and their libraries should acquire it.

The individual worker who confines himself to a single aspect of the sheep may find it of only ephemeral interest, and it is too expensive for that. It will prove even more expensive if he injects either of the two doses of adrenaline quoted incorrectly on page 33 from two quite separate sources. Each is a 1,000 times too large.

Brian Greenwood

Optics and Its Uses. (Modern University Physics Series.) By G. F. Lothian. Pp. 218. (Van Nostrand Reinhold: New York and London, September 1975.) Cloth £7.50; Paper £3.85.

Derived from a lecture course, this book is an undergraduate text which includes mention of modern developments such as lasers, non-linear optics, holography, integrated optics, Fourier transform spectroscopy, fibre optics, spatial filters, and so on, as well as dealing with basic theory. In a text of this length it is impossible to cover this very wide range of topics in depth, and reference to some is almost in note form. Although the consequent large number of section and sub-section headings break the flow in places, the arrangement should be helpful to a student, enabling him to make easy reference to a particular topic. In proceeding to an honours degree it would be necessary, as the preface suggests, for a student to supplement his reading, and most chapters refer to specialised books. The reader is expected to work through examples which are contained in relevant places throughout the book. As the title indicates there is emphasis on optical instruments and equipment; and understanding these is helped by a large number of good drawings, photographs, and tables. The latter illustrate a particularly useful feature of the book in providing an unusual amount of numerical data which would not otherwise be easily available to a student.

W. J. Bates

Books brief

Lord Rutherford on the Golf Course. By F. G. Mann, Trinity College, Cambridge, UK. Pp. 33+6 plates. (Published privately.) £1.50, UK; $4.00, Overseas and US. Copies from the author.

An accumulation of anecdotes, most, but not all, about Rutherford and golf. An absolutely bizarre story about Aston and a rice pudding. Nicely produced, but present-day prices hardly help such ventures even when published privately. □

Applied Geophysics. By W. M. Telford, L. P. Geldart, R. E. Sheriff and D. A. Keys. Pp. xvii+860. (Cambridge University: Cambridge and London, January 1976) £26.

The book is a very good one, written and illustrated with clarity. It sets out what is done in the present day in the application of geophysics to exploration. For each method the theory is given in sufficient detail for understanding that method, with the mathematical development to support it. Implementation of the method is described, with practical examples Further problems are given for the reader to solve, and each method has a good bibliography.

Two large sections of the book have special merit. A long chapter on the seismic method is a unified account of the sophisticated technology to which many have contributed time and money: it is almost a single line of development By contrast the chapters on electrical, electromagnetic and related methods bring together a great diversity of methods and practice under a common theoretical and descriptive treatment. Chapters on other methods are written with the same authority and clarity; the exception is, perhaps, the chapter on well-logging. This gives the impression of being an afterthought; and it suffers by comparison with the rest.

There are a few criticisms. The writers do not distinguish everywhere between interpretation and modelling, the inverse process. Time domain sampling at 4 ms intervals does not correspond with frequency domain sampling at 250-Hz intervals (p375). The noun 'practice' is not spelt with an s.

The book should fill the requirements of university teaching and of company training programmes It is of value to professional geophysicists who can rarely keep up with geophysical literature outside their speciality.

A. A. Fitch

An Introduction to Non-Numerical Computing. By Patrick A. V. Hall. Pp. 193 (MacDonald and Janes/American Elsevier Computer Monographs) £2.95.

This is a very competent collection of methods, algorithms and exercises divided into chapters on "Abstract Information and Algorithm Structures", "Machine Structures", "String Processing", "Files and Tables" and "Sorting". The book forms a reliable textbook of available techniques; it does not break any new ground but current thinking is well expounded. Hall's views on flow-charting are well worth noting. My only criticism is that a book on non-numerical computing should adopt a style different from mathematical texts. It is not that the mathematics is difficult—it has, in fact, been kept simple—but the flavour is still mathematical. References are also made to details of earlier chapters although the author says that the later chapters can be read in any order. The way in which the algorithms are presented also causes some difficulty, being a mixture of precise ALGOL-like instructions and English statements: but one soon adapts. The book is intended to support a second course in computing and leans heavily on ALGOL 60. Perhaps it could more usefully be based on ALGOL 68; it would appeal to a wider audience if it had been based on COBOL. The work is well illustrated and indexed. The exercises at the end of each chapter, although perhaps a little difficult for second course students, will be extremely valuable for teachers.

R. D. Parslow

obituary

Werner Heisenberg died on February 1, 1976. His youth was inspired; he lived through a long war which destroyed cities, lives and the spirit of people; in his old age he tried to rebuild a great tradition which had been virtually wiped out by Hitler.

The beginning of this century was the most remarkable period in the history of science. It was, in fact, so revolutionary that most people, including the majority of intellectuals, have failed to grasp what has happened. The leaders of this revolution were Heisenberg and his great teacher, Niels Bohr. The result was a complete explanation of the world of the atom. This fact is known. But the philosophical implications are all but forgotten. The philosophical principle is called 'Complementarity'.

Complementarity, or its older designation, dualism, is not new. It is as old as man. What are you: a piece of matter or a soul? It is difficult to deny etiher body or spirit. But to believe in both was considered sheer mysticism by a generation of scientists who lived in a world in which truth seemed to be simple.

It was Bohr who introduced a peculiar element into the most straightforward of all experimental sciences, physics. He based his quantum theory not on an explanation but on a contradiction. His ideas caused consternation, but his successes could not be denied.

Heisenberg, barely 24 years old, created order where there seemed to be chaos. Where Bohr navigated a new ocean keeping the shore of classical physics in sight by the use of the correspondence principle, Heisenberg left the old land and sailed beyond the point of no return. He found the New World of quantum mechanics. (It is even more remarkable that in inventing 'matrix mechanics' he did not know anything about matrices—he reinvented them.)

The unity of physics was reestablished by the cooperation of Heisenberg and Bohr. They asked the essential question: are atoms made of waves or particles? The answer: we cannot explain atoms without using both the wave picture and the particle concept. The two can be reconciled by setting limits to the accuracy in applying either description. For instance, the more accurately we know the position of a particle the less accurately can we know its velocity (or momentum). This is Heisenberg's 'Uncertainty Relation'. His first illustration was based on the resolving power of the microscope, a point on which, amusingly, he was ignorant at his PhD examination.

What has this to do with the body–spirit dualism? Heisenberg has shown that two obviously contradictory concepts, waves and particles, are not only necessary in explaining the structure of atoms but also, these two concepts can co-exist within a single rigorous mathematical formalism. If this can be done today for atoms why should it not become possible to do something similar someday in describing life or even a human being? Whether anything of the kind will happen one cannot tell. But Bohr and Heisenberg did something which even in the context of the great intellectual accomplishments of modern science is quite exceptional.

Werner Heisenberg, Photo: Associated Press

One often has the feeling that discoveries are just waiting to be made. Most scientists, even the great ones, merely accelerate science. They don't change it. Bohr and Heisenberg gave a completely new turn to the understanding of the world. Without them this understanding may never have come, at least not in our present culture. (This may sound like an exaggeration but I remember Bohr's statement: "If I can't exaggerate, I can't talk.") Without them the science of the atoms may have developed into a formalism even more remote from the common, unmathematical understanding than it is today. Without them the intricate concept of the interaction between the observer and the object may have been missed. (Heisenberg modestly denied this; he said that he only found what was there.)

Of course, the prime mover was Bohr. He was far ahead of everybody, but he was obscure, almost oracular. It was Heisenberg who brought the wisdom of Bohr (which Bohr managed to hide in his own words) to the level of lucidity where the common-or-garden theoretical physicist and mathematician could understand it, if he tried.

But in performing this translation of philosophy to exact science, Heisenberg did not lose any of the great original concepts. The philosophy (not coming from a philosopher) might have been lost. The science will survive. This is why Heisenberg is one of the few who have truly changed the world. It can never change back.

Heisenberg was made a professor when he was 26, and I became one of his students. We all talked physics, and played ping-pong and chess, and in this close environment a lot of fundamental work was done. Heisenberg received the Nobel prize in 1932 for the historic discovery of quantum mechanics and its implications, but much of his additional work would have sufficed in its own right to earn that distinguished honour. Some milestones are: papers on quantum field theory (QED with Pauli (1929, 1930) and divergence problems (1934)—Heisenberg's positivism can be exaggerated!), work on the solid state (the first understanding of ferromagnetism (1928)) and on symmetries (the distinction between ortho- and para-helium and hydrogen) and the invention of iospin (1932). We knew that the world was open and the key was reason.

Then came Hitler and reason was no more. In the summer of 1939, Heisenberg visited the US. Several of us asked him to stay. His answer to me was: "If your brother had stolen a silver spoon, he is still your brother." It was much more than a silver spoon. Both of us knew it, but neither of us could say so.

During the war Heisenberg worked on atomic energy. He used his position to save the lives of several fellow physicists. In his project he got nowhere. Why was Heisenberg unsuccessful in his wartime job? I am sure that the reason was not any lack of ability. I believed firmly that the obstacle was the silver spoon.

In his last years he wrote the story of his great experiences in science and the story of the tragedy of the deeply troubled times in Germany. In his writings, it becomes clear why Heisenberg failed during the war. He was happy to see that the technical difficulties were enormous and he concentrated on peaceful reactors. He only hoped that the American scientists, including the refugees from Europe, would come to the same conclusion. In 1941 he visited Bohr to give him this message. But Bohr's ears were closed and Heisenberg knew that he must continue to live in a small island surrounded by the Nazi horror; the greater world was too far away; even his best friend had turned his back. Bohr later escaped to the US, but Heisenberg's message was never delivered.

I shall not forget one of Heisenberg's recollections. With a colleague, he is hurrying through burning Berlin, trying to get back to two of his sons when his shoe catches fire. And while he puts out the fire, he discusses with his friend how science will be rebuilt in Germany when the catastrophe has run its course.

Shortly after the defeat of Germany, he hears of Hiroshima. He can hardly believe it. He is convinced that a political movement, or country, must be judged by its methods, not by its professed aims. (Heisenberg never mentions a nightmare which must have occurred to him: what would have happened if atomic bombs had been used against Germany. Hitler, unlike Hirohito, would not have had the fortitude to surrender. Hitler wanted Germany destroyed if the war was lost.)

But at last peace came and the reconstruction started. Heisenberg returned to the land he loved most, Bavaria. He continued to work on the hardest and most exciting problems of physics. The same basic philosophy that had led him to his formulation of quantum mechanics took him to the S-matrix approach to particle physics (1946). He later devoted himself to a bold attempt to understand particle physics through non-linear field theories with an inherent 'length' scale—continuing, in fact, pre-war ideas. This expressed his opposition to the idea that there are never-ending hierarchies of successively more fundamental particles— the 'big fleas–lesser fleas' syndrome. He also initiated the German programme for the production of nuclear energy.

In a long, magnificent and difficult life he never lost his sense of purpose, nor a sense of humour. On the 800th anniversary of the Bavarian state, he appeared on television and said: "The Bavarian unites the discipline of the Austrian with the charm of the Prussian." Most particularly, he retained a sense of balance. To each serious argument, he attached its opposite. He understood, from his own physics that between 'yes' and 'no' there are possible answers, less abrupt and more fruitful.

I am one of the relatively few who had a teacher like Heisenberg. From his life there remain for me two lessons. One is that the cataclysm of yet another world war must be avoided. Next time, though man will surely survive, it will be even more difficult to imagine how the spirit can be resurrected. The other is that the path of peace is not only difficult but also uncertain. No simple proposal, neither power nor appeasement, will suffice.

The title of Heisenberg's memoirs *Der Teil und das Ganze* means literally the part and the whole: the world cannot be divided into science and politics, nor into any other components. It is strange and wonderful that this lesson of unity should have been taught by a physicist who worked on a subject that most consider remote from the common understanding.

Edward Teller

Reports and publications

Great Britain

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nature

April 22, 1976

[illegible] Chemistry Library
SCIENCE COLLEGE
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CALCUTTA

Don't sit on the milestone

It is with some sense of relief, even pleasure, that it can be reported that the United States and the Soviet Union have actually managed to conclude an agreement on limiting underground nuclear tests to yields of less than 150 kilotons. Two months ago we were gloomily predicting that the superpowers would not meet their modest deadline of March 31, and we were wrong. In the process of negotiations it looks as if some real headway has been made towards establishing the principle of on-site inspection so important to the Americans, worried about cheating, and so tiresome to the Russians, worried about espionage. It has been agreed that the explosives for peaceful purposes shall not come in quantities of greater than 150 kilotons separately, although several may be fired simultaneously. Inspectors will monitor adherence, presumably by some down-hole device attached to each explosive to measure yield.

The satisfaction is that the threshold treaty, despite everything, is intact and the superpowers have done what they said they would. The vigour of the Soviet Plowshare programme, in which projects as big as the diversion of rivers still feature, has always been a worry to those who saw a treaty and a peaceful programme as incompatible for arms-control purposes. The neat formula by which this worry has been resolved offers some hope for future developments within the same framework.

This week's Geneva conference on test-ban monitoring will no doubt grudgingly acknowledge the Soviet-American agreement, but participants will also go on to say, privately if not in public, that

- Bilateralism is too cosy
- 150 kilotons is too high
- efforts to lower the threshold must go on.

Both superpowers have been vigorous in their high yield weapon-testing in the run-up to the treaty. In the United States advanced development research and testing has been put off for the last year and a half, according to ERDA's congressional supporters, while work was directed towards preparing for the treaty. There is thus a great danger that now that a milestone has been reached, the superpowers will sit on it. What better than that the Swedes, the Japanese, the Canadians and all other countries which have tried for years to make the superpowers see that vertical proliferation is as offensive as horizontal proliferation, should urge a painless formula gradually to reduce this threshold to zero. Such as:

$$\text{Threshold} = 150(1 - (\text{Year} - 1976)/10) \text{ kilotons.}$$

And how about some positive support for their efforts from the United Kingdom which has too much in recent years adopted a don't-rock-the-superpowers'-boat attitude engendered by nuclear subservience to the United States?

Mixture as before

Anyone who thought that, because of the prominence given to the fight for the Labour leadership, the consequences of Harold Wilson's departure might entail a different if not more favourable orientation in the UK government's disposition towards science, can be forgiven for feeling frustrated. Under James Callaghan the government has already twice indicated that, in this respect at least, continuity (meaning no change) is the order of the day.

The first, crucial indication came, not with Denis Healey's Budget, but in a White Paper released the same day entitled *Cash Limits on Public Expenditure* (Cmnd. 6440, HMSO). The limits, which apply to more than three-quarters of central government spending not counting social security benefits, are controls on the actual cash that may be spent in the year 1976–77 by specified blocks within government departments. The system supersedes the traditional budget estimates, which subsequent supplementary estimates used to inflate to compensate for escalated prices and costs during the year; now, if prices rise too high, the White Paper says, government purchases would have to be cut back.

The figures make the position clearer. The cash limit on Department of Education and Science's Science Budget, for example, which finances the activity of the Research Councils, is £226.1 million. The voted figure, announced earlier this year, is £215.949 million, and this remains the total available to the Research Councils. The difference represents an amount which could conceivably be available if times became really hard and the needy Research Councils could present a convincing case, to both the Minister and the Treasury, for the created margin of flexibility to operate in their favour. The Department of Energy's expenditure on nuclear energy, to take another example, has a cash limit of £124.4 million, and the same strictures will apply.

What is less clear is how the cash limits are arrived at. They obviously incorporate a government estimate of the rate of inflation for the year—an estimate that is still (temporarily?) secret. But if the declared aim of 10% by the end of 1976 is not achieved, research backed by the councils may not go ahead. And, given the need to contain public expenditure, if the rate ends up lower than forecast, the cash limits could conceivably be reduced.

The other, less crucial indication of a no-change policy, came with Mr Callaghan's Cabinet reshuffle. A glance tells all: Fred Mulley stays at Education and Science, Tony Benn stays at Energy, Eric Varley stays at Industry, Roy Mason stays at Defence (the only major department which has just a single block—of £5,835 million—covering all its expenditure). Peter Shore moves to the mis-named Environment giant.

It is early days yet, but with junior positions unlikely to make much difference, science policy looks for the moment like staying stagnant at the top. ☐

Keeping our heads about our heads

Controversial questions are currently being raised about psychosurgery. Keith Oatley of the Laboratory of Experimental Psychology at the University of Sussex comments on some of the issues

IN THE last few weeks public notice has been drawn to the use of physical and surgical interventions in the treatment of "mental illness". The release of the film "One Flew Over the Cuckoo's Nest", in which both electro-convulsive treatment and lobotomy were portrayed as techniques of punishment and control, was followed by a television documentary of a woman who had bouts of violent behaviour, and who was operated on by a neurosurgeon to remove part of her hypothalamus. The results were then reported of an Inquiry which upheld allegations made at a "typical" British mental hospital that electroconvulsive treatment was given "casually" and on "a disturbingly large number" of occasions. All this comes at a time when the Royal College of Psychiatrists is planning a controlled study of psychosurgery on 200 patients in the London area.

Under these circumstances it seems appropriate to consider some important issues raised by psychosurgery: the attempt to control entirely mental and behavioural symptoms (with no organic pathology) by physical stimulation or lesioning of the brain. Two fairly extreme positions can be taken up, both with a good measure of enthusiasm, righteous indignation, and often some reluctance to examine the evidence.

One position is that psychosurgery is barbaric, infringes patients' rights, and produces irreversible damage to the brain (how can less brain be better than more?). What justification is there in any case, it is asked, for supposing that people who have difficulties in living are "ill" or need to have some part of their brain stimulated or removed?

At the other extreme some members of the medical profession claim that psychiatric disturbances are illnesses with physical causes, that it has been shown that for some of these, intervention in the brain produces alleviation of symptoms, and even cures, and that doctors are in a position both to recommend, carry out and evaluate such treatment. (This latter point for instance was the conclusion of *The Times* medical correspondent Dr Tony Smith. "Only another doctor can be certain that a line of medical treatment was inadvisable, unethical, or simply wrong".)

Neither position squares well either with our physiological, psychological or sociological understandings of psychologically disturbed, or disturbing behaviour. Three kinds of consideration seem to be most relevant. First, there are the reported results of psychosurgical procedures which have already been undertaken. Secondly, there is evidence from experimental animal work (from some of which the psychosurgical techniques were originally derived), and from experimental analysis of, for example, accidental brain damage in people. Finally, there are some fundamental considerations of how people, including doctors and patients, ought to behave towards one another, backed if necessary with legal sanctions. It is worth considering one illustrative example from each of these.

In a recent issue of the *British Journal of Psychiatry* (**128**, 226–240; 1976), N. Mitchell-Heggs, D. Kelly and A. Richardson of St George's Hospital Medical School report on 66 patients given psychosurgery, and followed up over a mean period of 16 months. The operations were multifocal. Lesion-making electrodes were placed sterotactically in 10 sites of each brain (typically three in each frontal lobe, and two in each cingulum). The thermocoagulative lesions were aimed at various limbic tracts, and each estimated at 6 mm in diameter. Before and after operation patients were assessed on an impressive number of scales and inventories, and in 28 cases by a semi-independent assessor (a retired psychiatrist). The report was that 73% of the patients were improved at 6 weeks and 76% at 16 months. The paper is excellently detailed by the usual standards of such reports. The authors take pains to discuss the prerequisites for the operation (that is, an exhaustive trial of less radical treatments), and give a lot of statistics about their assessments.

Various comments can however be made. There is no control group, for example, and with the best will in the world the assessment is done by people who have a strong interest in reporting success of the procedure. Also, although one gets the impression that many patients did benefit substantially from the treatment (various remarks being made about "returning to work" and so on), the measures of improvement did not include assessment of whether the patients were improved with respect to their own objectives in life, or with respect to whether they were easier to contain in institutional care. Moreover, many of the scales used were heavily biased. Clinical rating scales, for instance, had three categories for "improved", one for "unchanged" and one for "worse". It is known that to approximate to fairness, rating scales should be symmetrical about the null hypothesis of "no change".

Regarding the experimental evidence (mostly from animals), this indicates that brain anatomy is no less variable than the anatomy of human faces, that sterotaxic placement of electrodes is in any case subject to errors (for example, of electrodes bending or being misdirected and thus ending up in places other than those planned). Even disregarding these there is no evidence that parts of the brain are related in a one-to-one fashion or any other simple way to categories of behaviour such as aggression or obsessionality. (The best review of these matters in the context of psychosurgery is by E. Valenstein, *Brain Control* (Wiley; 1973)).

Finally, although careful consideration of the experimental evidence from animal work would dissuade one from attempting psychosurgery, it does already take place, and some doctors believe it benefits some extremely disabled people considerably. If psychosurgery is to take place at all, what we do need is a proper trial, with proper controls, with informed consent of all subjects, and the participation of people (other than medics.) who know how to design and assess such experiments.

The trial should compare a group of patients who have been referred for psychosurgery (on the basis of severe disablement and failure of previous treatments). Half should have the psychosurgery, and the other half a further attempt at the best available non-surgical treatment (perhaps a combination of chemotherapy and behaviour therapy). All participants should be given supportive psychotherapy and rehabilitation help throughout. Assessment before and after treatment should be undertaken by several independent people, of different psychiatric persuasions, and without knowledge of what treatment each patient is given.

There are ethical problems; but these are less questionable than the current practice of doctors carrying out research without informed consent of all subjects in ill controlled studies which continue to leave room for doubt about the efficacy of procedures which then continue to be practised. ☐

Science and the media

David Fishlock, Science Editor of the *Financial Times*, looks at two recent publications* on a controversial relationship

SOME YEARS ago, while writing a science feature for a daily newspaper, I was advised by one scientist to ring up another for help on one specific point. But the professor in question, unknown to me, was not willing to talk on the 'phone and suggested I write. I said—perhaps impatiently—that I wanted to develop and verify the point right away and "can't wait three months for a reply". The professor would not budge but did ring my Editor to complain about my "rudeness".

A year or two later I met the professor at a Press conference called by her university to discuss the results of Government-sponsored work in which she had been closely involved. On that occasion she was helpful on everything connected with the Government-funded part of the work, but could not be drawn at all on the (very considerable) commercial implications of her university research. It turned out that she was also advising a large private company.

The readiness with which this university scientist was willing to place part of her knowledge and experience beyond the reach of the public (including her fellow scientists) is a good illustration of the scope for confusion about the respective roles of scientist and journalist. For those scientists who are interested in what the Press and broadcasting channels do with science —and there can be few research workers in these straitened times so unworldly as to be indifferent to the media's activities—these two short books, of identical title, offer a valuable grounding. One is the report of a joint scientist-journalist study group set up by the British Association (BA); the other, a contribution to OUP's excellent Science and Engineering Policy series.

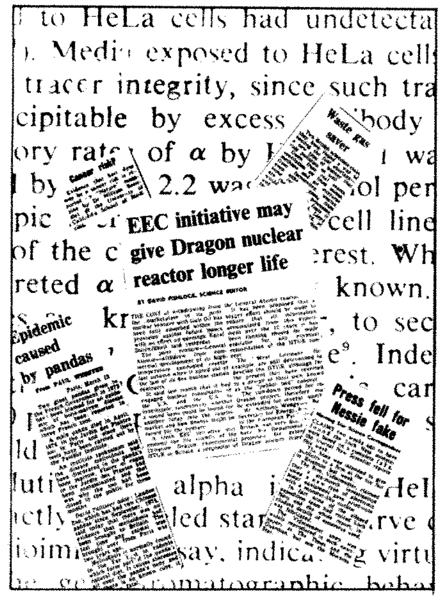
The BA report deals specifically with the quality of the presentation of science subjects to a wider public, improvement in which the BA believes calls for "a much closer relationship between scientists and journalists than exists generally today". It acknowledges that "several factors limit the frankness between scientist and journalist", among them contractual obligations and fears that the information may be over-dramatised. "It is not that he mistrusts the good science correspondent, who can be expected to present issues in a fair and balanced way, but that he fears any airing in the media runs the risk that the story will be taken up, sensationalised and distorted by others".

In 16 pages the BA report sets out very lucidly what the science journalist or broadcaster is trying to do, and why, what handicaps he must work under, and why he is so dependent upon the cooperation of scientists—and good scientists to boot. It has a salutary word of warning about "scientists, mostly with a gift for entertaining the public, who are tempted to exploit the media for personal reasons", and whose ability to dazzle a non-scientific audience may lead them to make "wild and reckless statements". It advises the media to be as wary of such scientists as they are of political adventurers. The corollary of course, is that if the genuine scientist will not help the journalist, he may well be tempted to make greater use of charlatans.

The BA study also touches upon the so-called growing public disenchantment with science, evidence for which it finds is thin. But it suggests that some journalists who believe in it allow their writing to reflect hostility towards science, and thus help to foster hostility between the public and science.

Dr Peter Farago's book covers broadly the same territory at about five times the length. The author is a scientist who edits technical journals and who thus has a toe in both camps. He tends to take a gloomier view of relations between scientists and journalists than the BA working party, which seems to reflect the views of those science correspondents on whom he has relied for first-hand experience of the craft. "The need for scientists to explain was a constant accompaniment to all discussions, together with the hurt accusation that they will not be properly questioned by reporters".

Farago notes that only one newspaper in Britain carries a daily item on science. This is the *Nature-Times* Science Report, modelled on the paper's famous Law Reports, from which I once culled a painfully funny item entitled "Raving and gobbling". There is little danger, however, of anyone doing himself an injury while reading one of its science reports, which are workmanlike but quite uninspired precis of, usually, original scientific papers, tucked away in a "ghetto" at the back of the paper and the bottom of the page.


EEC initiative may give Dragon nuclear reactor longer life
Epidemic caused by pandas
Press fell for Nessie fake

The *Nature-Times* Science Report is a good illustration of some of the major faults and failings of science writing and attitudes towards it today. It is "ghetto journalism" inasmuch as it caters for a select group of readers. Farago found no one on newspapers who found the reports readable—a strong condemnation of any regular newspaper item. They cry out for the touch of a sympathetic editor who knows how to write a good headline, how to inject some flair into the flat prose—all of which can be done, I should assure the purists, without distorting the facts.

But all the skills of the most sympathetic sub-editor could not overcome the handicap of having a guaranteed if inconspicuous place in the paper each day, which insulates it from the need to compete for space and attention. As a consequence it becomes isolated from the substance of all other reporting, no longer part of the fabric of everyday news. This may even redound in attitudes towards other science stories which get less attention because the paper thinks it is already doing its duty towards science by allocating a corner each day.

Neither of these two studies reach any very profound conclusions. They agree that science writing can be very good but often is not, and that it would probably be in everyone's interest if scientists and those who write about their work were to take a more sympathetic view of each other's activities. The seven conclusions set out in the BA report offer sensible advice for improving relations, and include the idea of more closely studying just what the public knows and thinks about science and where its ideas are coming from. □

**Science and the Media.* (British Association Publication 76/1 : London, March 1976).

Science and the Media. (Science and Engineering Policy Series.) By Peter Farago. Pp. 95. (Oxford University : London, April 1976).

WEST GERMANY

WEST GERMANY'S Economics Minister, Dr Hans Friderichs, recently announced that Bonn and Moscow had scrapped joint plans, first mooted some 18 months ago when Chancellor Helmut Schmidt visited Moscow, for the construction of a huge nuclear power station at Kaliningrad (formerly Königsberg) on the Soviet-Polish border.

Under the customary sort of no-cash-involved deal favoured by the USSR, Kraftwerk Union and other West German firms would have built the reactor with West German money (reportedly over £300 million), and given the USSR both the technology and the means of supplying electricity to its industrialised west; the USSR would have paid in the form of deliveries of electricity to West Berlin, which is surrounded by East German territory.

The reason given by Dr Friderichs for the cancellation concerned the USSR's proposals for pricing the electricity, which West Germany's electricity companies regarded as unacceptably high. But the suggestion that the project be abandoned came from the USSR when Dr Friderichs visited Moscow, and it is clear that the high prices sought by the Soviet Union in fact merely ensured the collapse of a deal to which East Germany had taken strong exception because of the inclusion of West Berlin.

The East Germans objected to the idea of greater security for West Berlin implied by the supply of electricity to the city. West Berlin may now have to construct conventional plants, although there is a plan for an underground nuclear reactor, while the Soviet Union, for the moment at least, will not obtain its 1300 MW power station.

- The recent State visit to West Germany by Egypt's President Sadat, widely regarded as further cementing relations between the two countries, included a highly publicised excursion to Germany's large nuclear power plant at Biblis. The possibility that Egypt will be a purchaser of German nuclear technology is not being discounted, particularly as Egypt's Minister for Science, Research and Nuclear Energy, El Guebeily, when he visited Germany at the end of January, was accompanied by the President of the Egyptian Academy for Research and Technology and the Deputy Director of the Egyptian Atomic Energy Authority.

Contracts arranged under the German-Brazilian agreement on nuclear cooperation of June 1975 have meanwhile been signed. They provide for uranium production, processing and enrichment as well as for the construction of nuclear reactors and of a reprocessing plant.

- Closely following publication by the European Commission of its second environmental programme for 1977 to 1981, in which priority is given to reductions in water pollution as well as reductions in waste and noise abatement, a group of West German environment experts has argued in a report presented to the Bonn government that a clean-up of the Rhine river is "primarily a German task" because the greatest share of the pollution is of German origin.

The group also suggests that the cost is "not too high" at about £300 million a year, arguing that the Federal government could bear one-third of this, state and local government could finance another third, while the industries discarging effluent into the river would pay for the remainder. The group recommends an effluent tax for industry to encourage Rhine-based companies to purify their own waste.

The report itself says thermal pollution and heavy metal pollution are not problems, but it does say that Rhine pollution has worsened, with salt and oxygen content often at unacceptable levels. In the week the report was published, however, it was announced from Paris that the environment ministers of Germany, France, Holland, Luxembourg and Switzerland (countries drained by the Rhine and its tributaries) had agreed on steps to reduce the excessive levels of salt.

The proposed scheme, which would start in two years if final agreement is reached, would cost about £6 million, but the ministers were unable to concur on how the running costs (around £450,000 a year) should be shared.

- "To strengthen the reciprocal information and communication process between State and Industry in the sphere of practical promotion of research and technology, and to offer starting points for a constructive critique of State measures". That is the aim of a document on state-aided research, entitled *Förderfibel* ("promotion primer"), published recently by the German Ministry of Research and Technology and drawn up by the Institut für Systemtechnik und Innovations-forschung (ISI). Directed particularly to small and medium size companies and to groups of researchers and inventors, the document has a preface by the Minister, Hans Matthöfer, which sets out a recommended procedure for budding inventors and research workers seeking support. The idea is to reduce the waste of time both by themselves and by officials they will approach. It is the ministry's first-ever detailed guide to government support for research.
- The chemical cycle is turning upwards in West Germany. The country's three major chemical concerns—Bayer, BASF and Hoechst—have all now reported a pick-up in business, matching exactly a similar recovery in Britain. As in Britain, the industry has been through a very depressed period, so much so that the upward trend in domestic trade will need to continue and begin revealing itself in foreign trade too if overall activity, including research, is to attain the sort of levels to which this huge and important sector of German industry has grown accustomed.
- Scientists, as much as anyone else in West Germany's academic community, are concerned about the implications of the tradition known as *Berusverbote*, under which the political reliability of people can be tested before they are admitted into the service of the state. Among the best documented cases in respect of higher education, most involve teachers in the social sciences. Estimates of the overall number of cases of refusal of appointment in the Civil Service go into the hundreds, and the number of screenings into hundreds of thousands.

The academics' concern is understandable: not only does it involve questions of basic human rights; it also affects them directly because they, like members of the regular public administration, are civil servants. The idea behind the practice is to prevent "extremists" taking up public appointments, but the major parties disagree less on the idea itself than on whether debarment should be determined merely by a person's membership of a party hostile to the Constitution rather than the particulars of each individual case. The difference has prevented agreement in the Upper House on the so-called Extremist Bill.

ISLAMIC SCIENCE

Scientists gather in Riyadh

Some two hundred Muslim scientists met at the University of Riyadh in Saudi Arabia last month for the Islamic Solidarity Conference in Science and Technology. Michel Chodkiewicz of La Recherche *reports*

A SCIENTIFIC renaissance in Muslim countries is one thing. An Islamic scientific renaissance is another. The former has begun, more or less successfully, in several countries, the latter remains to be done . . . if it has any meaning at all. For many Westerners, and for some Muslims this last point is far from being proved

This doubt was manifestly not shared either by those scientists present in Riyadh for the "Islamic solidarity conference in science and technology", or by the Saudi authorities who had bestowed munificent patronage on the meeting. Several Saudi ministers participated and King Khaled presided at the opening session. For all of these there was no conflict between Islam and science, either in the principles ("Seek out science, be it as far away as China", the Prophet had said) or in the facts (the brilliance of the abbasid era was witness to that).

But complacent contemplation of past glories must give way now to serious planning for the future, the majority of the presentations and discussion during the conference were very down to earth. From problems of nuclear energy, naval construction and scientific documentation to those of higher education, the subjects tackled seemed to have two priorities that of a better balance within the Islamic world between financial resources and manpower; and that of a very powerful drive for research and development, without which the Muslim world will never reach the "critical mass" above which it will be taken seriously scientifically, economically and politically.

The first of these problems—that of finance and manpower—is particularly evident in Saudi Arabia with its crying lack of manpower. (During our stay the government announced that civil servants were henceforth permitted—even encouraged—to work for private enterprise in their spare time) One of the unofficial aims of the conference was to profit by this gathering of scientists and to organise a reverse braindrain. Amongst the 185 Muslim scientists present, 27 worked in the USA, and it was particularly to them that a call was addressed to come and pursue their work in the Islam world. But researchers or technicians working in the resource-poor manpower-rich Islamic countries (such as Pakistan and Egypt) were also tempted by the salaries offered in the oil states.

In the long run this brain-drain, internal to the Islam world, may well be dangerous. The balance between the "umma" interest (the Islamic community as a whole) and those often divergent interests of constituent states will be difficult to get right Moreover, the scientists working in the non-Islamic world will only return to a satisfactory environment, stimulating professional contacts and so on. The creation of centres of excellence could help resolve this problem: to this end, a first step will be taken quite quickly, thanks to the founding of an Islamic Foundation for Science and Technology, probably at Taif, the Arabian city with the most pleasant climate, which has the added advantage of being near both Jedda, where a huge university is being built, and Mecca, site of another planned university.

On most aspects of the arrangements between Islamic countries, visitors, not representing their countries officially, could only express wishes However, the creation of a permanent Conference Secretariat, entrusted to the direction of the Rector of Riyadh University, will doubtless lead to some concrete action in the fields of, for instance, of documentation and scientific information (unifying the technical vocabulary, publishing scientific periodicals, organising seminars and symposia) and in collation of statistical data needed for planning.

Amongst the Conference's recommendations was the establishment of a consultative body comprising scientists and engineers working in military research and armaments. This initiative gave further substance to the already announced decision that several Arab countries had begun to develop a military-industrial community in Egypt. But to move out in this field from the Arab to the Islamic world will not be without its problems. It is worth noting that in his shrewd and much applauded speech at the final assembly Professor Fazlollah Reza, who was at the conference as a physicist but who is also Iranian ambassador to Canada, made remarks on all the resolutions adopted . . . with the exception of that concerning military consultation, which he passed over in silence.

● **Salah Galal writes:** A total of nine "Scientific Sessions" discussed specific issues. These included natural resources; industrial planning and growth; light and heavy industries and, specifically, chemical industries and manufacturing processes; university education, including its responsibilities towards industry, and technical education and training; research in universities and research centres; and translation and documentation.

It was three special "Projects Committees" which proposed the establishment of the Islamic Foundation for Science and Technology and the establishment of an Islamic Institution for the development of manpower, and produced the recommendations concerning armaments and military research.

RECYCLING

Paper chase

A recent publication from Friends of the Earth, *Ecological Paper Buying,* featuring a variety of recycled paper, urges the extensive substitution of waste paper for pulp as the raw material for paper making, in order to reduce Britain's huge annual pulp import bill. More waste paper, it says, could be used in newspapers, writing and printing papers and tissues, which currently take up 45% of the market.

The FOE spotlight a vicious circle, in which there is little demand for recycled paper because it is not widely available or insufficiently publicised, but not more widely available because there is little demand In fact the matter is more complicated than that As the Waste Management Advisory Council in the UK said in its first report last January, there would have to be an increase of 50% in the usage of waste paper by the early 1980s (compared to 1973–74) to save £100 million a year on imports given existing technology. The value of those imports was £270 million in 1974.

That means an extra 1.1 million tons of waste paper a year, which has to be collected and stored. The problems this presents, coupled with the problems of cyclical demand, seem to make a buffer stock necessary. One estimate of the finance involved in an excess stocks scheme is £10 million—a sum that is apparently not around at the moment ●

correspondence

Genetic engineering

Sir,—In recent months, molecular biologists have discussed the dangers which can arise from careless or ill-advised experiments in genetic engineering, and have attempted, at various meetings, to lay down guidelines as safeguards against these dangers. Since experiments in this field will, for some years to come, be possible only at specialised centres associated with drawing up these guidelines, their voluntary observation provides a degree of protection against dangerous experiments. The reports of such conferences, however, suggest an air of complacency, doubtless unintentional, giving the layman, or the scientist in other disciplines, an impression that all is under control This is dangerous.

Experience shows that it would be utopian to expect that such safeguards will continue to be accepted by all workers in the field. If it appears possible to use genetic engineering to cause damage or disruption for military purposes (several such possibilities can be envisaged), there will be organisations and states willing to sponsor and scientists willing to undertake such research, motivated by intellectual curiosity, or mistaken patriotism, or even a desire for gain and power. Under the circumstances of secrecy which would almost certainly be imposed, voluntary controls would be ineffective

Fortunately secrecy in research is rarely absolute and is difficult to maintain in the face of informed vigilance If the dangers of biological warfare appear to have receded in recent years, it is largely because of the vigilance of organisations like SIPRI, and the discussions stimulated by their reports. When everyone can recognise a danger and can work on counter measures, the danger becomes less acute.

It would thus appear to be an opportune time for molecular biologists to set up their own vigilance machinery, not as a police activity which would be repugnant to most scientists, but as information groups, which collect information on all known ongoing research, and can discuss and publicise the implications and the dangers of such work. Periodical publication of such reports in journals of general scientific interest would create an awareness and interest in these problems, and the publicity might discourage many persons who might otherwise be tempted into clandestine research.

In the present situation, the scientists' only safeguard against misuse of knowledge is the development of informed public opinion. Creation of such opinion should therefore be accepted as a responsibility of those who are exploring newer fields of research

Yours faithfully,

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Bombay,
India

Superstar technologies

Sir—I am very pleased that you considered our report on "Superstar Technologies" worthy of serious attention. Such constructive criticism as yours is important for any subsequent discussion of the report and its recommendations, and also helps improve our work.

The main point at issue between us is this does technology, like science, need peer-review and mutual criticism? We think so; you doubt it. What is our alternative? Apparently not very much, since you are suspicious of any serious restraint (even if only moral) on a technologist's freedom of action, lest he emigrate or his enthusiasm be snuffed out.

The working party was well aware of this point; you will find a reference (No. 27) to the paper by Freeman Dyson where it is argued eloquently. But they and the Council felt that the scope and power of modern technologies are so enormous, particularly in their political, economic and social factors, that self-monitoring by enthusiastic experts is simply not good enough. Hence our introducing the idea of "Superstar Technologies" and hence our advocacy of a Technology Implications Commission with its carefully delineated functions.

Yours faithfully,

Michael Swann

Council for Science and Society,
St Andrews Hill,
London, UK

Isolative sound-change

Sir,—It may be suggested that the most important unsolved problem of Historical Linguistics is: why do isolative sound-changes take place? The nature of isolative sound-change is best explained by an example Anglo-Saxon *bāt* changed into Middle English *bǭt* (to rhyme with *fort*), which changed into Modern English *boat;* Anglo-Saxon *hām* changed into Middle English *hǭm*, which changed into Modern English *home* It is possible to add very many examples with Anglo-Saxon *ā* to these two, and, on the basis of these examples, we formulate the sound-changes "Anglo-Saxon *ā* changes to Middle English *ǭ*, which changes to Modern English [ou]" (where [ou] is the vowel-sound in *boat* and *home*, spelt in different ways). This change is not affected by surrounding consonants —some changes are—that is why it is called "isolative".

The reason for sound-changes of this nature is entirely unknown. In fact, rather few suggestions as to a reason have been made; there was, perhaps, more interest in the subject in the nineteenth century than there is to-day. It may be that the problem, if it ever is solved, will be solved from disciplines other than Historical Linguistics—Psychology comes to mind. It seems that self-analysis is unlikely to help. I have an example of an isolative sound-change in my own speech: I pronounce the word *fire* identically with *far* (and so do some other people), whereas many people pronounce it almost to rhyme with *buyer*. And so for all the rhyme-words (*tyre*, *mire*, etc.). This change—[aiə] to [a·] in phonetic terms—has certainly taken place in my speech during my lifetime. But I am not able to say why

Yours faithfully,

Alan S. C. Ross

Southwick,
Sussex, UK

A hundred years ago

The Rhind Lectures on Archaeology, in connection with the Society of Antiquaries of Scotland, will be given by Dr Arthur Mitchell, commencing on Tuesday last, and continued on the following Fridays and Tuesdays. There will be six in all, and the subject is, "Do we possess the means of determining scientifically the condition of Primaeval Man and his Age on the Earth?"

from *Nature*, **13**, 495; April 20, 1876

news and views

Apollo asteroid Helin

from David W. Hughes

CONSIDERABLE excitement was caused by the discovery in January of asteroid 1976 AA because it has subsequently been found to be the first asteroid whose mean distance from the Sun is less than that of the Earth Eleanor Helin (California Institute of Technology) discovered the object on photographic plates taken with the 18 inch Schmidt telescope of the Palomar Observatory on January 7. It was then a faint 14th magnitude object in the constellation of Gemini. Using 46 observations taken between January 7 and February 3 Brian Marsden (Smithsonian Astrophysical Observatory) calculated the orbit of the asteroid and this is shown in Fig. 1.

On page 691 of this issue of *Nature*, Morrison, Gradie and Rieke of the Lunar and Planetary Laboratory of the University of Arizona report that the asteroid has a mean diameter of 900 ± 200 m and a visual geometric albedo of 0.20 ± 0.07 So Helin becomes the smallest asteroid ever to have its diameter measured accurately. (The asteroid will not be spherical but will have an irregular shape rather like that of Phobos, a satellite of Mars.)

A radiometric technique was used to obtain the diameter and albedo. The principle of the technique is simple. A fraction A (known as the albedo) of the total sunlight striking the body is reflected whereas the rest is absorbed The reflected radiation appears as the visual brightness, whereas the energy absorbed by the body (assuming the body is in thermal equilibrium and non-volatile) is re-emitted as thermal infrared radiation. The ratio between the reflected and re-emitted radiation gives the albedo. Combining this albedo with either the visual or the infrared brightness of the object gives the radius. Unfortunately there a few snags. All the infrared radiation cannot be measured from ground-based observatories as the 8–14 μm and 17–28 μm windows of the Earth's atmosphere must be used Second, the infrared emissivity of the soils and rocks on the small airless asteroid must be assumed as must also the dependence of the albedo on the solar zenith angle and on the wavelength of the incident energy. The rotation of the asteroid causes the unilluminated hemisphere to radiate some part of the absorbed solar radiation at the expense of the radiation from the illuminated hemisphere. The mean temperature of the observed disk is thus decreased by an amount dependent on the rotation period and the thermal inertia of the surface, leading to an over estimation of the radius. Morrison (*Icarus*, **19**, 1; 1973) finds that at wavelengths greater than about 10 μm the flux is sensitive to rotation and that all the above factors lead to a 10% uncertainty in the radius and a 20% uncertainty in the albedo

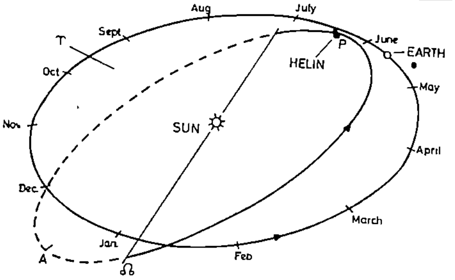

Fig. 1 The orbit of Helin (*IAC Circular*, No. 2915, February 24, 1976). Helin is shown at perihelion (P) and the position of the Earth at this time is also indicated. Helin will reach the aphelion of its orbit (A) on November 9 this year. (γ is the first point of Aries.)

Spectrophotometric studies indicate that asteroid Helin has a surface which resembles that of stoney, silicaceous meteorites and not a carbonaceous chondrite as previously reported (*Sky and Telescope*, **50**, 158; 1976). Silicaceous surfaces seem to be common among Apollo and Amor asteroids.

Helin is the 20th Apollo asteroid so far discovered and joins her more distinguished cousins Icarus, Geographos and Toro in this group of asteroids characterised by having perihelia inside the Earth's orbit.

Shoemaker (CalTech) points out that asteroid Helin is also one of the easiest places to get to in our Solar System apart from the Moon. It makes a most attractive target for an unmanned space mission. Asteroids—half way in mass between meteorites and planets—probably are typical of the building blocks which coalesced to form the planets. Not suffering from convective differentiation and geophysical and atmospheric effects as the planets did, they are most probably in the form of the original condensate from the pre-Solar System nebula and as such furnish an outstanding clue to the origin of the Solar System ☐

Plasmid exclusion and incompatibility

from J. R Saunders

MANY bacteria carry self-transmissible plasmids that characteristically inhibit infection with the same or related plasmids. This phenomenon of superinfection immunity is the result of the presence of two genetically and physiologically distinct barriers, surface (entry) exclusion and incompatibility

Surface exclusion is probably a device to prevent unnecessary mating within a population of homologous male cells Exponential cultures of bacteria carrying a transmissible plasmid are therefore poor recipients in matings with donor cells carrying a homologous sex factor. Exclusion is believed to involve recognition by the recipient of a specific (homologous) sex pilus on the donor and result in blockage of the transfer of DNA. It is mediated, at least in part, by the *traS* gene which forms part of the transfer operon of the F factor (Helmuth and Achtman, *Nature*, **257**, 652; 1975; Willetts, *J. Bact.*, **118**, 778; 1974). But the *traS* product is required only in the recipient and not the donor for surface exclusion to occur The observation that disruption of the mucopeptide layer of donor cells, abolishes the surface exclusion specified by the R plasmid R1*drd*19, whereas similar disruption in recipients has no effect, (Beard and Bishop, *J Bact*, **123**, 916, 1975), is therefore surprising Beard and Bishop suggest that surface exclusion is a barrier to exit of DNA from donor cells rather than, as has previously been assumed, to entry of DNA into a recipient It is presumed that a component (termed ML factor) associated with and dependent on integrity of the mucopeptide in the donor interacts with the *traS* product from the recipient. This leads to prevention of either establishment of stable mating pairs or mobilisation of DNA for transfer Transport of the *traS* product to the donor must presumably occur extracellularly by diffusion or by way of the conjugation bridge established during mating. Detailed molecular studies of the cell envelope of male cells should now help to elucidate the precise mechanism of exclusion.

Assuming that a superinfecting plasmid successfully enters a recipient it may then be incompatible with the resident. Plasmids which are incompatible do not necessarily exclude each other. Incompatible plasmids fail to coexist stably in the same cell and one or other is unable to replicate normally, being eventually diluted from the population. Incompatibility also operates in the case of the F factor whether or not the resident plasmid is inserted into the chromosome (Hfr).

Plasmids have been commonly classified on the basis that their incompatibility indicates a close degree of relatedness Over twenty (in)compatibility groups have been defined (see for example, Chabbert *et al.*, *J Bact*, **112**, 666; 1972; Datta and Hedges, *Nature*, **234**, 222; 1971; Grindley *et al.*, *Molec gen Genet*, **119**, 287; 1972) Members of a group are incompatible with each other but almost always compatible with members of other groups. Furthermore, the phylogenetic relationships established by compatibility grouping have been confirmed by studies of base sequence homology on plasmid DNA (see for example, Falkow *et al.*, *J. gen Microbiol*, **85**, 65; 1974; Grindley *et al.*, *J Bact.*, **115**, 387, 1973).

The mechanism of incompatibility is obscure and complex It may be associated with the process whereby the number of plasmid copies per host chromosome is regulated Some plasmids, for example the F factor, are present as about one copy per chromosome in *E coli* whereas others are present as multiple copies Jacob *et al.* (*Cold Spring Harb Symp. quant Biol.*, **28**, 329; 1963) proposed in the replicon hypothesis that bacterial chromosomes and plasmids both require specific maintenance sites on the cell membrane for replication and/or segregation Incompatibility and regulation of plasmid copy number could therefore be explained by competition for a limited number of plasmid-specific sites This explanation is less satisfactory for multicopy plasmids, which also exhibit incompatibility (Smith *et al.*, *Molec. gen. Genet*, **129**, 229; 1974), unless either the number of specific sites is high or one master copy of the plasmid responsible for governing replication and segregation binds at a single site Furthermore, does F incompatibility in Hfr strains mean that the Hfr chromosome occupies the F site or the chromosomal site, or both? To explain such anomalies it has been proposed that plasmid-specific repressors exist which regulate plasmid copy number. Some support for this comes from studying mutant plasmids which are present as more copies per chromosome than normal (copy mutants). Uhlin and Nordstrom (*J. Bact.*, **124**, 641; 1975) found that copy mutants of the R plasmid R1 fall into two classes. One showed decreased incompatibility with a test plasmid and is assumed to produce a defective repressor of plasmid replication. The other exhibited increased incompatibility due presumably to a defective operator, resulting in increased levels of free repressor. Other mutant plasmids have been isolated which show decreased incompatibility (Inc$^-$) and allow autonomous replication of superinfecting plasmids (see for example De Vries and Maas, *J. Bact.*, **115**, 213; 1973; Wyman and Novick, *Molec. gen. Genet.*, **135**, 149; 1974). These mutants are however chromosomally inserted. Mutations in chromosomal genes can also affect incompatibility (see for example, Bezanson and Iyer, *J. Bact.*, **123**, 137; 1974).

Hiraga (*Proc. natn. Acad. Sci U.S.A.*, **73**, 198; 1967) now reports the isolation of novel F′ factors that can replicate autonomously in Hfr strains of *E. coli.* These plasmids carry a region of the *E. coli* genome including the *dnaA–bglA* region and a specific site designated *poh*$^+$ (permissive on Hfr). Since such F′ factors effectively bypass the normal *inc*-mediated blocking of plasmic replication, Hiraga has proposed that the *poh*$^+$ site represents the origin of replication (*oriC*) of the chromosome of *E. coli. poh*$^+$ presumably functions by allowing these F′ factors to use *oriC*, instead of the F origin of replication (*oriF*) which is blocked by the *inc* product(s) (repressors?) in Hfr strains. It will be of interest therefore to see whether two autonomous F′ factors both carrying *oricC* are compatible. This may determine whether F incompatibility is qualitatively different when the resident F particle is autonomous as opposed to integrated into the host chromosome (Hfr). Complementation studies of mutants of F defective in vegetative replication have been hampered in the past by incompatibility. Therefore the means of circumventing incompatibility afforded by Hiraga's F′ factors may aid in the elucidation of the genetic control of plasmid replication.

Masters (*Molec gen Genet.*, **143**, 105; 1976) also reports the construction of F′ factors carrying the chromosomal region between *bgl* and *mtl* including the origin of replication. It may be significant that this region includes *dnaA*, one of the genes essential for the initiation of chromosome replication. Strains made diploid for the origin of replication by such F′ factors grow more slowly than normal and have a reduced DNA/mass ratio This is to be expected if the ratio of chromosome origins/cell mass is disturbed since this is thought to be involved in controlling initiation of DNA replication (Donachie, *Nature*, **219**, 1077; 1968). Such strains could be used to determine whether control of initiation of chromosome replication is positive or negative It should also now be possible to map the precise location of the origin on the genome of *E. coli.* F′ factors carrying the origin should therefore prove valuable in understanding the mechanisms of both plasmid incompatibility and the control of DNA replication. ☐

Ribosomes and secretory proteins

from A. Amar-Costesec

EUKARYOTIC cells contain two distinct populations of cytoplasmic ribosomes: some are free in the cytosol, others are bound to membranes, which thus appear "rough". Cells secreting proteins very actively have a large proportion of their cytoplasmic ribosomes bound to the membranes of the endoplasmic reticulum (ER). In hepatocytes, which synthesise and export serum albumin and other plasma proteins, 60% of the RNA is present in membrane-bound ribosomes and only 20% in free ribosomes The attached form of ribosomes is predominant in pancreatic acinar cells and in plasmocytes, secreting digestive enzymes and immunoglobulins, respectively In the mid 1950s, Palade suggested that membrane-bound ribosomes are the site of synthesis of proteins which are to be secreted.

When tissues are homogenised, the ER membranes rupture under the action of shearing forces, but then reform as vesicles. The portions of the ER studded with ribosomes are transformed into rough vesicles studded with ribosomes on their external face These rough vesicles retain the ability of ER to incorporate amino acids into proteins. Using biochemical and morphological methods, it was demonstrated that ribosomes bind to membranes through the larger of the two ribosomal subunits, the smaller being attached only to the larger (Sabatini *et al.*, *J. molec. Biol*, **19**, 503; 1966) and that the nascent polypeptide chain is vectorially discharged into the intraluminal compartment of the ER (Redman *et al*, *J. biol Chem.*, **241**, 1150; 1966), probably as an extended chain moving through a tunnel in the large ribosomal subunit. The phospholipid membrane forms a barrier between the cytosol and the intraluminal space.

Blobel and Dobberstein (*J Cell Biol.*, **67**, 835, 1975) propose that ER possesses binding sites in the form of receptor proteins in the membrane. Specific sites on the receptor proteins interact with corresponding sites on the large ribosomal subunit. The receptor proteins form a second tunnel which extends through the thickness of the membrane and is linked to the tunnel of the large subunit. The vectorial discharge of the nascent polypeptide would occur through the two tunnels, resulting in segregation of the protein within the intraluminal space. The main problem, however, is the mechanism by which the cell decides which protein must be segregated, or alternatively how an RNA entering the cytoplasm is able to choose between free or membrane-bound ribosomes.

In 1971 Blobel and Sabatini formulated the 'signal hypothesis' which proposes that mRNAs for secretory proteins possess, after the initiation codon common to all mRNAs, a series of signal codons. When the mRNA is translated by a ribosome, the polypeptide chain then contains at the amino-terminal end a sequence of amino acid residues called the signal sequence The length of this sequence is estimated at 20–25 amino acids, corresponding to 1,000 to 2,000 daltons. When the nascent polypeptide chain emerges from the large ribosomal subunit, it is recognised by the receptor proteins of the ER membranes, which then form the transient tunnel through the membrane. In the intraluminal compartment, the signal sequence is removed by an endopeptidase. Folding of the peptide chain possibly occurs after this post-translational cleavage. The newly synthesised protein is then ready to follow the pathway of exportable proteins through various cell components (such as Golgi, condensing vacuoles and secretory vesicles).

One implication of the signal hypothesis is that exportable proteins are synthesised as polypeptides larger than necessary, as a result of the presence of the signal sequence. Different authors working with heterologous cell-free systems have observed that the product of *in vitro* translation of mRNA for immunoglobulin light chain is larger by about 20 amino acids than the authentic light chain. Similarly, it has been shown that mRNAs from calf parathyroid glands are translated by a wheat germ extract into preparathyroid hormone which migrates more slowly in sodium dodecyl sulphate–polyacrylamide gel than parathyroid or proparathyroid hormones. Here also, the translation product is larger than the normal protein. Another implication of the signal hypothesis is that significant homology, if not complete identity, may be expected in the signal sequence of the polypeptide chains of several exportable proteins. Devillers-Thiery *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 5016; 1975) extracted mRNAs from dog pancreas rough microsomes, and translated them *in vitro* in an artificial system. The polypeptides formed were separated by polyacrylamide gel electrophoresis into five "regions". Substantial homology was found in the sequence of 16 amino acid residues at the amino-terminal end. This sequence, which contained predominantly hydrophobic amino acids, could represent the metabolically short-lived signal sequence of dog pancreas secretory proteins. Another compelling argument is given by the work of Schechter (*Proc. natn Acad. Sci. U.S.A.*, **70**, 2256; 1973) and of Swan *et al.* (*Proc. natn. Acad. Sci. U.S.A*, **69**, 1967; 1972) who have shown that in mouse myeloma, mRNA coding for the light chain of immunoglobulin is larger than that required to code for a native light chain. It is tempting to believe that the additional nucleotide mass could, at least in part, represent the signal codons.

One advantage of the signal hypothesis is that it resolves the old problem of two functionally distinct populations of ribosomes. All ribosomes are equivalent and whether they become attached to ER membranes or remain free in the cytoplasm depends on the mRNA they are translating. The mechanism proposed for secretory proteins may be applicable to other "segregated" proteins, such as the hydrolases of lysosomes, the oxidases of peroxisomes, and some mitochondrial and chloroplast enzymes. Thus, the signal hypothesis could throw light on the mechanism of biogenesis of complex organelles which contain segregated enzymes. □

Amorphous p–n junctions: curiosity or breakthrough?

from Andrew Holmes-Siedle

THE annoying feature about semiconductor electronics is that we are so frequently forced to use single-crystal forms when it would be ideal if we could instead rapidly deposit thin films of semiconductor on an inert, cheap substrate such as silica glass and perform the usual topological processing on these films (for the many advantages of this method, see, for example, Holmes–Siedle, *Rep. Prog. Phys.*, **37**, 699–816; 1974). Unfortunately, without epitaxial growth conditions, deposited films are always either polycrystalline or amorphous. The grain boundaries in the former state make it impossible to fabricate many kinds of device while the main bar to the wide use of amorphous semiconductor films has been our inability to form p–n junctions in them by doping.

Doping in semiconductors is the introduction, often in complex patterns, of impurity-containing regions, in order to modify resistivity and conduction type and form rectifying structures. In the crystalline silicon lattice; for example, a phosphorus atom, having a valence bond to spare, contributes electrons to the conduction band. Until recently, it seemed that this effect was always negated in the amorphous network by the large number of free bonds which could accommodate the space valence bonds of a dopant. But Walter Spear and co-workers at the University of Dundee, working with silicon films deposited from a glow discharge in silane gas, found that these films would respond to the presence of dopants, although they were still non-crystalline by all the usual criteria. Now they report the first preparation of a p–n homojunction structure in a completely amorphous material (Spear *et al*, *Appl. Phys. Lett*, **28**, 105–107, 1976). This was achieved by beginning the deposition of the silicon film in a stream of silane gas containing a little phosphine and then changing the phosphine to borane, or *vice versa*. The devices which were formed had quite good rectifying characteristics, with 'on-off' current ratios which appear to be about a hundred. The 'on' resistances of fairly large-area devices were still quite large by commercial standards (I would estimate them to be of the order of 10^4 ohms for a square millimetre of junction area) but this might be reduced with further work.

The key to the success of Spear's group probably lies in the careful studies which they previously undertook on the physics of amorphous silicon which included the development of a field-effect measurement of the density of states in the mobility gap against potential energy, thereby making it possible to monitor the variations of this fundamental property while the preparation conditions were varied at the fancy of the investigator. Other aspects of the energy-level spectroscopy of these systems also produced useful guidance as to how nearly the mobility gap and Fermi level corresponded to those in single-crystal material of the same doping. 10^{17} phosphorus atoms give a conductivity of 10 $\text{ohm}^{-1}\text{cm}^{-1}$ in crystalline silicon but only 10^{-4} $\text{ohm}^{-1}\text{cm}^{-1}$ in these films and boron seems to operate at a similar level of inefficiency in doping, implying that the characteristic ability of the amorphous network to negate the doping

Standardisation in human cytogenetics

At the Fourth International Conference on Standardisation in Human Genetics, held in Paris in 1971, a Standing Committee was appointed to examine problems relating to standardisation in human cytogenetics and to make recommendations thereon between international standardisation conferences.

During the past four years the Standing Committee has been responsible for publication of the Report of the Paris Conference and a supplement to that report. In addition, the Standing Committee sponsored a workshop on human cytogenetic registries, held in Edinburgh in April 1975 (*Nature*, **256**, 456; 1975) and on the recommendation of this workshop, appointed an international advisory committee on cytogenetic registries.

The terms of reference of the Standing Committee call for elections to a new committee no later than the International Congress of Human Genetics in October, 1976 and the possibility of holding the next standardisation conference at that time. For these reasons the committee has recently circulated recommendations to the participants of the Paris Conference, which have been approved as follows: (1) That a standardisation conference on the lines of the Paris and Chicago Conferences could not be justified in 1976 and, therefore, that no such conference should be held in conjunction with the Fifth International Congress of Human Genetics in Mexico City in October, 1976, (2) That a Standing Committee charged with the task of monitoring developments relating to standardisation and nomenclature in human cytogenetics should be maintained; (3) That an open meeting of all interested human cytogeneticists should be held at the Fifth International Congress of Human Genetics in Mexico City in October, 1976. At this meeting a report of the Standing Committee would be received and a new Standing Committee elected. The existing Standing Committee will be acting as a nominating committee and will be preparing a slate of nominations for consideration by the meeting in Mexico City. These nominations will take into account regional and sectional interests to ensure, so far as possible, a fully representative Standing Committee.

Suggestions for nominations should be sent to the Standing Committee. The time and place of the Mexico City meeting will be announced later and circulated to those requesting this information. Nominations and requests for further information should be addressed to: Dr John L. Hamerton, Department of Genetics, Health Sciences Children's Centre, 685 Bannatyne Avenue, Winnipeg, Manitoba, R3E OW1 Canada.

effect is still prevalent, since roughly 999 out of 1,000 dopant atoms still seem to be suppressed. It remains to be seen whether this apparent inefficiency in doping can be improved on. The important discovery, however, is the fact that even a few dopant atoms are not suppressed. Junction action is thereby produced and a new device technology may even be in the making. The extent to which this technology could compete with single-crystal semiconductor technology depends less on the inefficiency of doping mentioned above, which can be countered, than on the achievement of low minority-carrier recombination-generation rates in the junction regions. High rates lead to high reverse currents in rectifiers and loss of efficiency in photovoltaic applications. It will be a much more challenging task to make these recombination rates suitably low but success in doing so could have a greater impact than the much-researched 'amorphous threshold switch' technology, which now seems to have lost any commercial momentum it ever had. An amorphous p–n junction technology would have much in common with the increasingly popular silicon-on-sapphire technology except that the serious restrictions in area imposed by the dimensions of sapphire crystals would have been removed and the way thus opened for integrated circuits as large as the page on which this column is written. ☐

Superfluid within superfluid

from P. V. E. McClintock

At last, somebody has gone out on a limb and named an encouraging number for the temperature of the anticipated superfluid transition among the ^{3}He atoms of a liquid ^{3}He–^{4}He solution. According to Patton and Zaringhalam of the Massachusetts Institute of Technology, writing in a recent issue of *Physics Letters* (**55A**, 95; 1975), the transition is likely to take place under conditions which can be achieved experimentally.

They are brave men. The first estimates of the superfluid transition temperature T_c of pure liquid ^{3}He were wrong (too high) by a factor of more than fifty. Experimenters eagerly sought the phenomenon, but failed to find it at the predicted temperature: the theorists then increased the sophistry of their calculations and revised their estimates of T_c downwards, a cycle which was repeated several times. This procedure was so utterly demoralising for those doing the experiments (being the classical brainwashing technique of repeatedly raising the victim's hopes and then dashing them again) that, when the superfluid transition finally did manifest itself in Osheroff's cryostat at Cornell, it took several weeks for him and his co-workers to realise the significance of what they had discovered. Hence the notable reluctance, so far, of most theorists to predict a T_c for ^{3}He–^{4}He solutions.

A solution of a few percent ^{3}He in ^{4}He is stable down to the lowest temperatures and is, even without the expected transition, of considerable intrinsic interest. The ^{3}He atoms exist and move in a background aether—the liquid ^{4}He—which is itself a superfluid whose transition temperature lies at the relatively high value of 2 K. At low temperatures, where the ^{4}He entropy is essentially zero, the ^{3}He atoms dominate the behaviour of the liquid. In fact, if one completely ignores the presence of the ^{4}He (except insofar as it results in each ^{3}He atom having an effective mass which is larger than the bare atomic mass), and simply treats the ^{3}He atoms as though they were an ideal gas, one gets the right answers for most of the liquid's properties.

Now, it is known that an attractive interaction occurs between the ^{3}He atoms of a solution and, at a sufficiently low temperature, this is expected to give rise to the formation of Cooper pairs, and hence to superfluidity. It is not yet clear whether the relative angular momentum of the atoms in a pair will be zero, as for the Cooper pairs of electrons in a superconductor, or unity as is apparently the case in superfluid pure ^{3}He. The situation would certainly be very peculiar in either case, however, since the superfluid ^{3}He which was formed would exist in another, completely separate but interpenetrating superfluid, the ^{4}He, which is fundamentally different in nature in that it does not acquire its superfluidity through the formation of Cooper pairs. The bizarre physical properties to be expected of such a system were discussed in considerable detail by Khalatnikov in his invited address (Khalatnikov, Mineev and Volovick

Proc. 14th. Inf. Conf. on Low Temp. Phys, North Holland Pub. Co., Amsterdam, **5**, 102, 1975) to the recept LT14 conference in Helsinki—but he was careful to avoid alluding to any numbers. In particular, he did not make any mention of a value for T_c

Patton and Zaringhalam have now described a theory which, with one adjustable parameter, gives a reasonable description of the pressure dependence of T_c for pure ^{3}He. They fix this parameter by fitting their theory to the experimental results already obtained and then, together with a variety of other experimental information, use it to derive a value of T_c for a ^{3}He–^{4}He solution. They conclude that, for a saturated solution of 8.85% ^{3}He in ^{4}He under a pressure of 20 bar, $T_c=0.9$ mK This result is some 10,000 times larger than an earlier prediction by Østgaard (*Phys Lett.*, **94A**, 433; 1974) of ~ 10^{-4} mK.

These calculated values of T_c may be compared with 0.7 mK, the lowest temperature to which pure ^{3}He has so far been cooled Making thermal contact to a ^{3}He–^{4}He solution is rather more difficult then to pure ^{3}He at these temperatures, however, so that cooling a solution below 0.9 mK may not necessarily be quite within present experimental capabilities; but it certainly cannot be far removed.

Remembering the bitter experience of the theoretical T_c values for pure ^{3}He, a certain level of scepticism is inevitable in relation to Patton and Zaringhalam's 0.9 mK It is nonetheless encouraging, and we should not have to wait very long for their prediction to be put to the test in an actual experiment. ☐

No need for glycophorin?

from a Correspondent

IT is a truism that any breakthrough in science, as in art, fashion or sport, quickly becomes a commonplace Nowhere is this more obvious than in protein sequencing However, although the complete sequencing of a protein no longer has, perhaps quite the same impact as previously, the sequencing of a membrane protein is still a relatively rare occurrence This has been achieved recently for glycophorin, a major protein of the human erythrocyte membrane (Tomita and Marchesi, *Proc natn Acad Sci. U.S A.*, **72**, 2964–2968, 1975; see *News and Views*, **258**, 478; 1975) Some of the sequence is familiar from earlier publications by Marchesi and his colleagues. In particular the now famous sequence of 23 amino acids lacking charged residues and enriched in hydrophobic residues such as leucine, isoleucine and valine, is placed firmly towards the carboxyl terminal of the polypeptide chain. This sequence is thought to be responsible for integrating the protein into the hydrophobic environment of the membrane. A segment of the polypeptide chain at the carboxyl end is believed to protrude internally whereas the amino terminal sequence, bearing a large amount of carbohydrate, is on the outer side of the membrane.

The function of this quantitatively important constituent of the erythrocyte membrane is unknown. Some recent results suggest that it may be dispensed with altogether without undue effect on the survival either of the erythrocyte or of the person carrying these cells.

The starting point for this work was the identification of a rare genetic variant in humans which affects erythrocyte surface antigens. The En^a antigen is present on the surface of almost all human erythrocytes except in some very rare individuals. Three families are known with this trait: two in Finland and one in England (Dainborough *et al., Vox Sang.*, **17**, 241–255; 1969; Furuhjelm, *et al., Vox Sang*, **24**, 545–549; 1973) These individuals, who are clinically normal, also show unusually weak activity of another common erythrocyte antigenic system called MN. It has been known for many years that MN antigenicity is associated with glycophorin and this prompted Tanner and Anstee (*Biochem. J.*, **153**, 271–277; 1976) to look for this component in En(a–) erythrocytes. It turns out that the erythrocytes apparently lack glycophorin completely. Erythrocyte En(a–) membranes analysed by SDS polyacrylamide gel electrophoresis have normal peptide composition except for the loss of glycophorin and a minor glycoprotein species of slightly smaller molecular weight.

Tanner and Anstee then tried to label glycophorin in En(a–) erythrocytes with lactoperoxides. In normal erythrocytes, glycophorin and another membrane protein are readily iodinated by this means. In En(a–) erythrocytes however, only one membrane component was labelled and this was not glycophorin.

What these results imply, therefore, is that glycophorin itself is not absolutely required for the function of the erythrocyte This is surprising on two counts; first, glycophorin is one of the two most abundant proteins of the erythrocyte membrane Together with a component called protein 3 it accounts for almost half of the membrane proteins. Second, there is considerable evidence that glycophorin and protein 3 form a non-covalent complex in the membrane. It has been suggested (see Rothstein *et al., Fedn Proc.*, **35**, 3–10; 1976) that the complex plays a coordinated role in anion transport Although protein 3 appears to be the more directly involved in anion transport, it will be interesting to see if the quite drastic changes in the composition of membrane protein of En(a–) erythrocytes result in any physiological effects on ion transport. ☐

Aspects of tumour biology

from M. J. Bevan and I. S. Trowbridge

The Armand Hammer Symposium in Tumour Biology was held at The Salk Institute, San Diego in January 1976.

THE origin of the cancer cell and its interaction with the immune system were the main themes of the Symposium.

In the opening session, David Baltimore (Massachusetts Institute of Technology) and Charles Heidelberger (McArdle Laboratory, Madison) discussed viral and chemical oncogenesis respectively. Baltimore showed that the resistance to infection by N-tropic or B-tropic murine leukaemia viruses conferred by the FV-1 locus is probably at the level of transcription implying that FV-1 is a regulatory gene coding for a repressor. Viral DNA could be made and integrated into the genome of resistant host cells but unlike the case of sensitive host cells virtually no viral RNA was produced. He also described the *in vitro* transformation of murine bone marrow and foetal liver cells by Abelson Leukaemia virus and its quantitation by a focal assay. Whether the target cell for transformation is a pre-B cell or a more primitive stem cell and how many such cells are present in the various haemopoietic organs is uncertain. It seemed unlikely that a B-cell itself is the target as no immunoglobulin-bearing cells were transformed.

Heidelberger established that chemical carcinogens actually transform normal cells rather than select pre-existing malignant cells from within the population of "normal" cells in his *in vitro* fibroblast cultures, by showing that frequencies of transformation greater than 50% are possible. He also described evidence that chemical carcinogens do not cause transformation by activating endogenous RNA tumour viruses. One importance of his experimental system is that it provides a screening procedure for potential carcinogens. In this regard it was interesting to hear that the effects of promotors, chemicals that do not themselves cause cancer but which potentiate the action of other carcinogens, can also be assayed in this system.

George Klein (Karolinska Institute, Stockholm) described the interaction of Epstein-Barr virus with human B lymphocytes. He described convincing evidence for the identity of EB virus receptor and the complement receptor of B cells stressing however that not all complement receptors bind the virus. He also showed experiments with EB virus-negative B cell lines and their *in vitro* transformed sublines which illustrated the selective *in vitro* growth advantage that transformation with EB virus confers on the transformed cell.

Gerald Edelman (Rockefeller University, New York), suggested that it may be possible to bring together some of the diverse changes which take place when a cell is transformed by considering the role submembranous microtubule assemblies have in maintaining cell morphology and transmitting extracellular growth signals to the cell interior.

Many tumours express cell surface structures which can be recognised as foreign by the immune system of the host. Ways in which genes in the major histocompatibility complex may control immune responsiveness were a major theme. J Klein (University of Texas, Dallas) showed partial amino-acid sequence data from four laboratories on various alleles of the 45,000 molecular weight H-2K and H-2D major histocompatibility antigens of the mouse. Comparison of the sequences of different alleles of one locus shows multiple amino acid replacements—30–40% of the positions are different. These are "complex allotypes" in Lee Hood's terminology though Klein warned that comparing products of other alleles with the H-2^{b} antigens could be misleading since this haplotype may have come from a different subspecies or different species of mouse than that from which other inbred strains were derived Comparison of the sequences of the products of the K and D loci again shows 20–40% amino acid substitutions Here the remarkable thing is the degree of similarity between their products, confirming their origin by gene duplication. The K and D sequences of the mouse appear more similar to each other than to either the human HLA-A or HLA-B sequences, that is, species-specific residues are shared by K and D. This raises the possibility that the two loci, K and D in mice and HLA-A and HLA-B in humans separated in evolution after divergence of the species.

What does this have to do with immune responsiveness? These gene products control interactions between cytotoxic effector T cells and target cells. Doherty and Zinkernagel have suggested that neoantigens (including tumour-associated antigens) are recognised only in association with K and D products as complex determinants (the altered-self hypothesis). Klein favoured the idea that the K and D restriction of cytotoxicity is explained by two independent interactions. First, the T cell receptor binds the neoantigen which only leads to a successful cytolytic interaction when the associated H-2K or D itself binds the identical K or D molecules on the target cell, a like–like interaction. Edelman felt that this was ruled out by his experiments with Schrader showing that antibody to H-2K and D blocked a cytolytic interaction only when the target (not the killer cell) was treated. He proposed a T-cell receptor with two subsites, one for K or D and the other for neoantigen leaving the origin of the restriction as "altered self".

H. McDevitt (Stanford University, California) described the distribution of H-2 I-region-associated antigens on various T cell populations. Neither precursor cytotoxic T cells, effector cytotoxic T cells, nor helper T cells are removed by treatment with anti-Ia serum and complement. In contrast, suppressor T cell activity in two systems (allotype suppression and suppression of a hapten carrier response) is sensitive to anti-Ia serum and complement. Partly on the basis of these results, McDevitt interprets the helper T cell replacing factor of Taussig and Munro, which is Ia positive and antigen specific, as being a suppressor T cell factor like that described by Tada The factor, according to McDevitt, enhances the IgM response only as a consequence of suppressing the IgG response

Despite this, McDevitt believes that Ir genes which determine high or low responsiveness to particular antigens, are expressed at the helper T cell level, that is, failure to develop helper T cell activity is not due in all cases to excessive suppressor T cell activity. Therefore the Ir gene product expressed in helper T cells is predicted to lack Ia determinants

A. Mitchison (University College London) was concerned with whether two immunogenic but separate molecules on the same cell would be coprocessed by the immune system. Some tumour antigens may be weak because the tumour cell presents no other determinants for associative recognition. He showed that the antibody response to the Thy-1.2 alloantigen could be enhanced by the associative recognition of other H-2 coded or non-H-2 coded alloantigens. Nylon wool purified primed T cells do help the anti-Thy-1.2 response of primed purified B cells and Mitchison has evidence that the T cells and B cells effectively cooperate when recognising different alloantigens on the same immunogenic cell. AKR (H-2^{k}, Thy-1.1) spleen cells which have been primed with CBA thymocytes (H-2^{k}, Thy-1.2) give a good response to CBA thymocytes on transfer into irradiated AKR recipients. B10.BR thymocytes (H-2^{k}, Thy-1.2) also stimulate a good secondary response but B10 (H-2^{b}, Thy-1.2) or F$_1$ (CBA×B10) thymocytes fail to generate a good response. This failure to stimulate cannot be interpreted as meaning that Thy-1 is seen by T cells as a modification of H-2 antigens, that is, as a type of altered-self hypothesis. Mitchison views this as an example of antigenic competition in which recognition of a foreign H-2 somehow removes the Thy-1 antigen (which has to be on the same cell) from the inductive processing system.

Antigen presentation in the response of rats to syngenic chemically induced tumours was a feature of R. Baldwin's talk (University of Nottingham, UK). An animal protected against a tumour by immunisation with irradiated cells shows responsiveness to individually specific tumour antigens. A tumour bearer, on the other hand, responds also to embryonic antigens common to many tumours (as detected in the lymph node microcytotoxicity test). Grafting irradiated tumour cells leads to tumour-specific protective immunity. However, if the host has been pre-exposed to tumour antigen in the form of isolated membrane or 3M KCl extracts then a subsequent graft of irradiated tumour cells does not lead to protective immunity although a humoral response is observed. This raises questions of how the class of the response to a tumour is determined.

Melvin Cohn (The Salk Institute, San Diego) made the interesting comment that all tumours presently used as model systems have already undergone rigorous selection for their ability to escape immunological rejection since they arise in immunocompetent hosts. He suggested that the complexity of tumour rejection may be more easily analysed by establishing tumours in immunoincompetent hosts from the *in vitro* transformed colonies of Heidelberger and studying the way in which these cells escape single step immunological selection pressures such as T cell killing or antibody-dependent cytotoxicity. □

review article

Archaean high grade complexes and modern continental margins

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Two kinds of geological terrain formed before 2,500 Myr ago. First, the greenstone belts, which are linear basins full of sediments and volcanics, possibly comparable with the marginal basins that occur behind modern island arcs. Second, and the subject of this paper, are high grade complexes containing rocks that have been deformed and recrystallised at high temperatures and pressures—granitic gneisses derived from granitic rocks are particularly common. Examples occur in north-western Scotland, Greenland, the Labrador coast, the Limpopo Belt in southern Africa, southern India, and probably the Aldan and Kola shields in the USSR. These complexes are so similar lithologically that broad generalisations about their tectonic evolution are justifiable. No major attempt has been made to see if they share any similarities with modern geological environments whose origins are better understood. We suggest that they are ancient analogues of the modern deep seated granitic batholiths located along the main arc axis of active plate margins of North and South America, for example in California and the Andes; accordingly, they are complementary to the greenstone belts in their back-arc setting.

THE tectonic evolution of Archaean high grade gneissic regions is poorly understood, especially the origin of tonalitic–granodioritic gneisses which make up 80–85% of the present surface[1,2]. Various source materials have been proposed, on the basis of chemical analogies; they include andesitic–dacitic volcanic rocks[3–6], modified earlier gneisses[7], dioritic rocks that underplated the crust[8], plutonic granitic rocks in general[1,9,10], and igneous tonalites and granodiorites[11]. Some local chronologies are known[9,10,11], but little is understood of their evolutionary meaning.

We propose a more general approach in which all the tectonic and chemical interrelations of the Archaean rock types are compared with properties of rocks in Mesozoic and Cainozoic fold belts, whose tectonic environments are better understood. Specifically, we suggest that Archaean quartzofeldspathic gneisses and their enclosed remnants of layered igneous complexes[12] and supracrustal rocks are metamorphosed and deformed equivalents of calc-alkaline batholiths in the Cordilleran Fold Belt of western North America, and probably of the similarly active plate margin of the Caledonian Fold Belt of Britain. A strict uniformitarian comparison cannot be expected because, first, the older rocks show indications of metasomatism, second, mineralogical reactions have occurred which indicate burial of some of the Archaean rocks to 20–30 km; third, earlier plates may be smaller, less coherent and more mobile than modern plates, resulting in a different tectonic style at continental margins, and fourth, early magmas produced by partial melting of the lower crust and upper mantle may differ chemically from modern ones Even at the risk of oversimplification, however, we have made the comparisons in order to provide ideas to be tested by further mineralogical, petrological and geochemical studies

Archaean relationships

The Archaean high grade regions, to which this model is most readily applicable, are north-western Scotland (the Scourian)[13], Greenland[1,10], Labrador[14], the Limpopo Belt of southern Africa[15], and southern India[16].

Gneisses formed at least twice in Greenland and Labrador, and only the younger are relevant here. In the other regions only the younger gneisses have been recognised so far. The relevant chronology is [1,9,10,14]:

Oldest	Formation of gneissic basement
	Deposition of supracrustal rocks (basic volcanics, pelitic schists and gneisses, marbles and quartzites)
	Interthrusting of supracrustals and basement
	Intrusion of layered ultramafic–gabbroic–anorthositic complexes
	Intrusion of tonalite–granodiorite
Youngest	Deformation and metamorphism of tonalite–granodiorite to give rise to high grade gneisses.

The Greenland gneisses grade into homogeneous 'granitic' rocks in low-deformation zones[9], and Labrador gneisses occur as deformed veins of intrusive 'granite'[11]. Gneisses in all regions have calc-alkaline affinities whether metamorphosed to amphibolite or granulite grade Accepting the arguments against a volcanic origin[8], we prefer to envisage a suite of tonalitic–granodioritic rocks as the precursor of the quartzofeldspathic orthogneisses[1]. The chemistry of major and trace elements in the gneisses compares best with that of 'granitic' rocks from

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modern continental margins[11], and the very low initial ratios of strontium isotopes (about 0.701) are consistent with direct derivation from the mantle[17].

Metamorphosed remnants of layered igneous complexes with an idealised upward stratigraphy of ultramafic–gabbro–anorthosite[12] occur within the gneisses. The complexes are bordered either by supracrustal rocks into which they were intruded[18] or directly by the gneisses[19]. Parts of the Fiskenaesset Complex, western Greenland, are associated with supracrustals rocks[20], and other parts are not[19]. The intrusion of the complexes was followed immediately by that of the 'granitic' rocks[9,10,14], resulting in the complexes occurring as giant rafts in vast quantities of 'granitic' rocks: thus all the Fiskenaesset gneisses, accounting for 84% of the present surface area[2], are younger than the Fiskenaesset layered complex[21]. Even where some older gneisses predate the complexes, as near Godthaab, western Greenland, the younger gneisses may account for over half the area[9]. Apparently the most highly developed gneisses coincide with the most highly developed layered complexes (for example, at Fiskenaesset).

The complexes are in all stages of fragmentation. In places, individual layers persist for many kilometres (for example, Fiskenaesset, Limpopo), but the complexes commonly occur as isolated lenses and pods from kilometre to metre size (southern India[22], western Greenland[23], Scourian[13]). Although late deformation was responsible for local folding, thinning and boudinage of some layers, it would seem an unlikely cause of the fragmentation of the complexes into the millions of small pods and lenses found in many areas. Perhaps this fragmentation was connected with the engulfment of the complexes by granitic material on an enormous scale. Explosive mechanisms may have been involved, and a detailed comparison of the Archaean occurrences with the appinitic rocks and breccias of the Caledonian mountain chain, especially in Donegal, is desirable[10].

Comparison with oceanic crust

Archaean sediments, volcanics and layered complexes have been interpreted as representing a section through oceanic crust[24]. Graphitic pelites and marbles have been correlated with modern pelagic sediments leading to the interpretation of quartzites and psammites as recrystallised oceanic cherts; the occurrence of dolomitic limestones at the Mid-Atlantic Ridge has also been noted. Amphibolites with occasional relict pillows have been matched with oceanic basic volcanics, and the anorthosite–leucogabbro–ultramafic sequence has been related to layered igneous complexes of the type inferred to occur under some modern oceanic basalts[25].

Objections can, however, be raised. Where the Archaean quartzites are best developed, as in the Limpopo Belt, they are current-bedded and thus interpreted as orthoquartzites (R. M. Shackleton, personal communication). The carbonate (dolomitic marble)–orthoquartzite–K-rich pelite (sillimanite mica schist) association, typical of most high grade regions, is best explained by deposition in a shallow-water, continental margin[26,27]. Comparison of the chemistry of amphibolites with that of volcanic rocks is fraught with difficulties because of chemical changes undergone during metamorphism[28]. Some Archaean amphibolites have chemical patterns similar to those of modern island-arc volcanics (B. E Leake and B. F. W., unpublished) and others to oceanic volcanics[29]. To distinguish the latter from the early tholeiitic series of island arcs is difficult, especially after metamorphism[30]. Anyway, low-K tholeiites occur in various tectonic environments, and distinction between oceanic and island-arc or continental volcanic rocks, so demonstrable in modern times, should be less apparent in the Archaean[31] Grab samples indicate that layered igneous complexes in modern oceanic crust apparently comprise predominantly Ti, Fe-rich gabbro, and contain only about 5% Cr-poor labradorite anorthosite and gabbroic anorthosite (leucogabbro)[25]. Archaean complexes are very different in both rock proportions and chemistry; thus gabbros are minor and not enriched in Ti and Fe, whereas the bulk of the bodies consist of bytownite–anorthosite and leucogabbro (where best preserved), often interlayered with chromitite Alpine-type ophiolite complexes derived from the oceanic crust are distinguished by a scarcity of the cumulate features characteristic of gravity settling, whereas many Archaean complexes contain chromitites, plagioclase cumulates and crystal-graded layers. Volcanic and plutonic rocks formed at mid-ocean ridges should be characterised by highly variable age relations, stratigraphic succession and distribution (J. Cann, personal communication), whereas Archaean rocks, especially in western Greenland and Limpopo, are noted for remarkable stratigraphic regularity and persistence along strike for tens of kilometres. Only a little acidic tonalite can be generated by one-stage fractionation of ultrabasic mantle.

We believe that these differences between recent oceanic rocks and Archaean complexes are so great that the latter cannot be slices of oceanic crust.

Comparison with Cordilleran fold belts

The Mesozoic batholiths along the axis of the Cordilleran fold belts of North and South America represent the roots of magmatic arcs related to palaeosubduction zones. The most abundant rocks are tonalite, granodiorite and quartz monzonite (adamellite)[32]. The content of K_2O generally increases away from the Pacific margin[33] although potassic granites are a minor component of the batholiths[34].

Within the Peninsular Range batholith of southern California, layered igneous complexes composed largely of calcic plagioclase and primary amphibole include a succession of minor peridotite and troctolite and predominant hornblende anorthositic gabbro[35]. The mineralogy and chemistry of these rocks is similar to that of the Archaean complexes. The California complexes have been interpreted as early cumulates of a hydrous and refractory nature precipitated from a basaltic–andesitic magma which later yielded the calc-alkaline batholithic suite.

Concentrically-zoned ultramafic complexes of the Alaskan type are clearly distinguishable from the alpine-type ultramafic complexes usually considered to be obducted oceanic crust and mantle. The Alaskan-type complexes, which also occur in the Ural Mountains, British Columbia, Venezuela and perhaps Japan, have been interpreted as the products of fractional crystallisation and flow differentiation of basaltic magma in deep-seated, volcano feeder pipes[36]. Crystallisation under hydrous conditions in the pressure range 2–11 kbar has been invoked to explain the presence of magnetite and hornblende as well as olivine, clinopyroxene and plagioclase. The associated eruptive products are andesites and probably aluminium-rich tholeiitic basalts.

Although few detailed mineralogic–petrologic studies have been undertaken, it is certain that calc-alkaline volcanic and plutonic rocks are linked with the hydrous, amphibole-bearing intrusions, typically developed along modern continental margins.

Many of the layered complexes within the Peninsular Range tonalitic batholith are bordered by a group of sediments (the Julian Schists) that comprise sillimanite mica schists (meta-K pelites), quartzites and carbonate rocks, an assemblage that is strikingly similar to that in the Archaean tonalitic gneisses and that likewise formed in a shelf–continental margin environment.

In the Dawros–Cashel area of Connemara, several fragmented layered complexes[37] with peridotite[38,39] and hornblende–calcic plagioclase gabbro grade upwards into plagioclase gneiss[40]. According to a plate tectonic model[41], this area lies on the northern continental margin of the Caledonian Fold Belt. We suggest that the petrochemical features of these bodies render them comparable to the Archaean and California examples. Particularly important is the generally accepted evidence of major horizontal movements—now represented by the extensive occurrence of nappes—together with the regional metamorphism to garnet and kyanite grades in the Dalradian rocks of Scotland[42]. These features occur in the high grade Archaean complexes[10], and apparently represent the final stage of tectonic activity at an active continental margin.

Mineralogical–petrological–chemical considerations

The mineralogy and petrology of the Archaean complexes is interpreted as the result of igneous crystal–liquid differentiation combined with deformation and regional metamorphism. The Fiskenaesset Complex provides the clearest evidence. Co-ordination of folded fragments has revealed seven major zones. From bottom to top they are: minor, magnetite-rich ultramafic rocks; leucogabbro with anorthosite laminae; gabbro; leucogabbro with marked cumulate texture; anorthosite; chromitite; and garnet anorthosite[20,43]. Hornblende occurs in all of the rock types. This sequence has been interpreted as the result of gravity differentiation of a water-rich basaltic magma in which the early crystallisation of Mg–Al–Ti–Cr–magnetite and the continued precipitation of primary magnesio- to tschermakitic-hornblende led to a trend quite different from that found in dry, layered basaltic complexes such as Skaergaard. Some megacrysts may locally represent original primocrysts, but most rocks have metamorphic textures superimposed on modal variations retained from the original igneous layering. Related structural and mineralogical evidence has been found in the Sittampundi (ref. 44 and B. F. W. and J V. S., unpublished), Limpopo[18] and Lewisian complexes[45], but the data are less complete for various reasons. Solid-state recrystallisation has modified mineral compositions in all of the complexes, and metasomatism has changed some bulk compositions.

Although estimates of the temperatures and pressures reached during metamorphism depend on somewhat controversial thermodynamic models and experimental phase equilibria, metamorphism of the Archaean high grade complexes certainly occurred at depths estimated at between 15 and 40 km. In South Harris, chemical relationships between garnet, pyroxenes and olivine indicate that granulite metamorphism occurred near 800–860 °C and 10–13 kbar (ref. 46). Garnet-clinopyroxene rocks from Sittampundi reacted near 850 °C and 7–8 kbar (ref. 47). Also at Sittampundi, the coexistence of staurolite, kyanite, garnet and other minerals in one hand specimen indicates conditions near 600 °C and over 5 kbar, whereas the occurrence of sillimanite-bearing assemblages indicates somewhat different conditions (B. F. W. and J. V. S., unpublished). Olivine and plagioclase grains have not been found together in a single hand specimen from Fiskenaesset, and they may have been replaced by spinel, pyroxene and amphibole. Adjacent grains of orthopyroxene and plagioclase coexist in some Fiskenaesset rocks without having reacted to garnet and quartz. Chemical relationships between finely intergrown oxide minerals (B. F. W., J. V S., I. M. Steele and F. C. Bishop, unpublished) indicate equilibration down to 300–600 °C, and those between intergrown sulphide minerals (F. C. Bishop, J. V. S. and B. F. W., unpublished) indicate equilibration down to 100–200 °C. The metamorphic history differs from one complex to another, from one part to another, from one mineral assemblage to another, and from one grain to another. In spite of the local idiosyncrasies, which depend on relative diffusion coefficients and stresses, there are overall large scale effects interpretable in terms of high grade regional metamorphism which must have affected the tonalite–granodiorite gneisses as well as the igneous complexes.

The extent of metasomatism is uncertain. In the Fiskenaesset Complex, mineral compositions vary monotonically with position[43], and erratic metasomatism should be insignificant except perhaps for minor development of mica and sodic plagioclase and of some minerals of the contact facies[20]. In the Limpopo Complex, one traverse showed strong erratic plagioclase zoning to more sodic compositions, whereas compositions were steady in another traverse[18] A major question is whether most of the amphibole in the Fiskenaesset Complex crystallised from the igneous magma or whether it is a metasomatic product after pyroxene. In some thin sections, amphibole occurs around pyroxene grains, but the intimate interlayering of amphibole-free and amphibole-bearing assemblages in many felsic and mafic rocks suggests an igneous origin before metamorphic recrystallisation. Although small composition differences between amphiboles from felsic and mafic parts of the same zone[43] probably result from metamorphic equilibration, the generally tschermakitic-pargasitic nature of all the amphiboles is like that of amphiboles produced synthetically from hydrous basaltic melts (see, for example, refs 48–51). Furthermore, tschermakitic-pargasitic hornblendes occur in modern unmetamorphosed rocks where the texture is consistent with the crystallisation of amphibole from a magma (see refs 52–54). Rare symplectites of spinel and pyroxene may result from the dehydration of amphibole[20]. Although much further study is needed, especially of relatively undeformed specimens from low deformation zones, we assume here that amphibole is one of the igneous minerals at Fiskenaesset, and that the magma was hydrous.

Magnetite occurs as dust in some mafic silicates at Fiskenaesset, but most magnetite of the lowest zone occurs as discrete Cr-rich grains whose association with ilmenite and Mg–Al spinel can be interpreted as the result of metamorphic decomposition of primary, Mg–Al–Cr–Ti-bearing magnetite (I. M. Steele, F. C. Bishop, B. F. W and J. V. S., unpublished). Its occurrence throughout the complex as a primary phase, especially in the early mafic zone, would explain why there is no strong trend towards iron enrichment. The primary precipitation of magnetite requires redox conditions more oxidising than those within dry basaltic layered complexes[55], and redox conditions for the Fiskenaesset magma could be inferred by reference to phase relationships in basaltic melts[56] Chromium-bearing spinel, loosely called chromite, occurs in many Archaean complexes. Its composition range—atomic Mg/(Mg+Fe) 0–0.4; Cr/(Cr+Al) 0.2–0.6—distinguishes it from Cr-spinels in alpine-type, concentric-Alaskan, and dry-basaltic layered complexes (ref. 18, and J. V. S. *et al.*, unpublished). The compositional adjustment of cumulus Cr-rich spinels by reaction with silicates and trapped liquid has been demonstrated in the Rhum layered intrusion[57]. No zoning was detected in any Fiskenaesset chromites in spite of considerable textural variation, and the general compositional features are independent of the modal proportion of Cr-spinel to silicates. Particularly noticeable is the absence of Cr-spinel in most, but not all, of the lowest ultramafic zone at Fiskenaesset, and its principal occurrence as thin layers between late anorthosites. Many basaltic intrusions have early chromite and late magnetite. The near-absence of early chromite at Fiskenaesset can be explained by the precipitation of Mg–Al–Cr–Ti-magnetite as a result of a relatively high state of oxidation.

Archaean high grade complexes carry highly calcic plagioclase (Fiskenaesset, mostly An_{96-80} (ref. 43); Limpopo, up to An_{75-80} (ref. 18); Sittampundi, up to An_{100} (ref. 44, and J. V. S. *et al.*, unpublished)) which distinguishes them from Proterozoic anorthositic complexes carrying labradorite and andesine. Even the latest plagioclase at Fiskenaesset is highly calcic (An^{80}), and there is no trend to very sodic or even potassic feldspars, as in many differentiated basalts. As proposed for the ultrabasic and basic intrusions of Connemara[37], the most obvious explanation for the persistently high Ca content of Fiskenaesset plagioclase is the continued depletion of Na and K from the wet residual magma by precipitation of primary hornblende with $\sim 2\%$ Na_2O, $\sim 0.4\%$ K_2O and $\sim 12\%$ CaO. Calcium-rich pyroxenes from dry basaltic complexes carry $<1\%$ Na_2O, $<0.2\%$ K_2O and $\sim 18\%$ CaO, whereas Ca-poor pyroxenes carry hardly any Na_2O and K_2O, thereby permitting the concentration of Na and K into later plagioclase.

In spite of all the uncertainties caused by regional metamorphism and deformation, the major mineralogical features of the Fiskenaesset complex seem to be qualitatively consistent with the crystal–liquid differentiation of a wet, oxidised, basaltic magma in a closed magma chamber. Detailed experimental syntheses along the lines of those already carried out on basaltic and ultrabasic compositions (refs 48–50, and 58–61), followed by subsolidus equilibration, are needed to provide detailed reference data. In the meantime, it is necessary to rely on qualitative and semiquantitative features (the early precipitation of Mg–Al–Ti–Cr-magnetite; late crystallisation of highly calcic plagioclase and high-Fe, medium-Cr spinel; prolonged crystallisation of tschermakitic–magnesio-hornblende; medium Fe/Mg ratio of ferromagnesian minerals) in looking for possible matches to the Fiskenaesset Complex in modern environments. To set the search in the widest context, modern rocks with highly calcic plagioclase need to be examined, and their wider mineral contents should be checked against the Fiskenaesset Complex.

A nodule from alkali-olivine basalt, Auckland, New Zealand, contains 74% An_{97} (ref 62). Ultramafic nodules in basalts from Hawaii and Itinome-gata, Japan, carry minor plagioclase ranging up to An_{90} (refs 63 and 64). Associated minerals are too highly magnesian to match those at Fiskenaesset. Some basalts from oceanic environments carry megacrysts or phenocrysts of composition An_{70-89} (refs 65–67), but the differentiation trend in these near-surface flows is quite unlike that in Archaean complexes. The younger volcanic rocks of Tonga, and the Cape Hoskins volcanic rocks, Papua, contain An_{80-90} phenocrysts[68,69], but titanomagnetite occurs only in the late differentiates; amphibole is absent.

In the Antilles Island Arc, phenocrysts up to An_{85} occur in andesite and basalt from Montserrat, along with amphibole-plagioclase (An_{93-89})-magnetite xenoliths[70]. At Soufrière Volcano, St Vincent, ejected plutonic blocks contain plagioclase (An_{96-89}), olivine (Fo_{79-67}), salite, hastingsitic amphibole and Mg–Al–Ti-magnetite[71]. The Soufrière amphibole has too little SiO_2, the magnetite has no detectable Cr, and the salite has too much Al_2O_3 to match the Fiskenaesset minerals. The Salt Lick Creek layered intrusion of Western Australia[72] contains plagioclase (An_{84}) associated with olivine (Fo_{81-84}), orthopyroxene (Fs_{15-20}), clinopyroxene ($Wo_{48-44}En_{46-48}Fs_{6-8}$) and Mg–Al-chromite, in assemblages similar to those in layered complexes in Rhum[73], the Cuillins of Skye[74], and Mull[75]. The absence of amphibole and magnetite distinguishes this suite from the Fiskenaesset Complex. Megacrysts of An_{87-93} with inclusions of augite or perhaps augite plus amphibole occur in feldspathic dykes of allivalite composition in Skye[76], and ultrabasic breccias in the Rhum Complex[54] contain plagioclase (An_{87-97}), olivine (Fo_{83-82}), augite ($Wo_{43-46}En_{46-48}Fs_{8-9}$: 4 weight % Al_2O_3), primary kaersutite (TiO_2, 4.9; Al_2O_3, 11.6; Cr_2O_3, 0.5; FeO, 8.2; CaO, 11.9; Na_2O, 2.6; K_2O, 1.0 weight %), phlogopite, Mg–Al–Ti chromite–magnetite and picroilmenite. Although the amphibole is too high in TiO_2 and low in SiO_2 and the augite is too rich in Al_2O_3, this mineral assemblage might resemble selected assemblages in the Fiskenaesset Complex after metamorphism of the former at about 5 kbar and 900 °C. The Greenhills Ultramafic Complex of New Zealand[77], ranges from chromite-bearing dunite to wehrlite, gabbro and allivalite, all three of which carry titanomagnetite and brown hornblende. The sequence of the opaque minerals provides a ready distinction from the Fiskenaesset Complex, but major-element compositions of the olivine, pyroxene and plagioclase (An_{88-92}) lie in the Fiskenaesset ranges

Alpine-type ultramafic complexes[78] can be dismissed because their olivines and orthopyroxenes are too magnesian rich (mg near 0.9) to match the range (0.86–0.74) for the Fiskenaesset Complex. Concentrically-zoned ultramafic complexes of Alaskan type[52,78,79] contain rocks with various amounts of primary hornblende, olivine, clinopyroxene, magnetite, chromite, ilmenite and hercynite spinel with compositions fairly similar to those of the Fiskenaesset minerals; but plagioclase is virtually absent except in late hornblende-plagioclase pegmatite, and the type of zoning differs from that at Fiskenaesset. The interpretation[36] of these zoned complexes as feeder pipes of andesitic volcanoes is feasible, and the resemblance of their mineral compositions to those at Fiskenaesset could be attributable to the crystallisation of a hydrous basaltic magma in both cases, one being open and the other closed Layered gabbroic and ultramafic rocks in a tectonic melange on the Kodiak islands of Alaska have received only a brief description[80], but they should be compared closely with Archaean complexes Ultramafic rocks at Emigrant Gap, California[81], are part of a mafic complex showing near-concentric zoning from dominant wehrlitic peridotite in the core to tonalite in the rim. Olivine, pyroxenes and plagioclase from the inner rocks lie in the composition range of the Fiskenaesset rocks. Magnetite is ubiquitous and chromite also occurs, but no compositions have been determined. Amphibole is absent in many rocks though it occasionally replaces augite. Detailed chemical studies are needed, but the differentiation trend to tonalite apparently results because the water content was too low to produce substantial amounts of amphibole.

The Fiskenaesset Complex fits best with the numerous bodies (~ 100) of peridotite-gabbro of up to 70 square kilometres in outcrop dispersed among the larger tonalite–granodiorite bodies of the Peninsular Range Batholith of southern California[82,83]. The mineral assemblages and compositions of the peridotites, gabbros and anorthositic gabbros[35] can be matched with those at Fiskenaesset except for small details, and the interpretation in terms of the differentiation of a wet basaltic–andesitic magma is similar. The differences between the two mineralogies are explicable in terms of different degrees of metamorphism and small variations in the differentiation trend resulting from small changes of water pressure, oxidation state, and so on.

Although more detailed mineralogical–petrological–geochemical studies are needed of layered complexes in Archaean and modern environments, our survey indicates that, first, Archaean complexes can be explained by the metamorphism of igneous complexes developed from wet basaltic magmas differentiating under oxidising conditions, and, second, the Archaean complexes can be matched chemically with the complexes in the southern California batholith. Mineralogical data for the layered complexes of the Caledonide fold belt of Ireland and Scotland are sparse (refs 37–39, 84 and 85) but the presence of calcic plagioclase, apparently primary hornblende, and medium-Cr, medium-Fe spinel are tantalising clues.

Discussion

The crux of our argument is that the late Archaean intrusive tonalites (now tonalitic gneisses and granulites) differentiated from a more basic, mantle-derived magma generated in some type of active continental margin. We suggest that enclosed, layered igneous complexes can be matched mineralogically with complexes enclosed within a tonalitic batholith of western North America, and that the associated Archaean and Mesozoic orthoquartzite-carbonate-K-pelite associations are both typical of a shallow-water continental margin. Furthermore, our argument that the Archaean high grade regions are equivalent to the deep-seated levels of modern arcs is consistent with the current interpretation of greenstone belts as fossil back-arc marginal basins[86,87].

Because Archaean tectonic conditions and magma compositions may differ from present ones, analogies must not be pressed too far. Whereas the granitic diapirs and vertical, near-cylindrical, zoned complexes of the Alaskan type reflect vertical transport in modern continental margins, strong arguments favour horizontal tectonics in the Archaean[10]. The apparently greater abundance of komatiitic lavas in Archaean times can be explained by a steeper geothermal gradient resulting from higher heat production by younger radioactive species. Early basaltic magmas may have been wetter than younger ones because the Earth was undergoing strong outgassing in the Archaean (see, for example, ref 88). Early plates may have been smaller, less coherent and more mobile Nevertheless, we suggest that the growth of continents in the late Archaean can be studied profitably in the context of models related to accretion at the leading edge of modern continental plates.

B. F. W. thanks the Geological Survey of Greenland and the NERC and J. V. S. thanks F. C. Bishop, I. M. Steele, NASA and the NSF. R. K. Nishimori and J. Hawkins contributed discussions and we thank P. J. Wyllie and R. C. Newton for reviews.

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Observation of light emission from a rhodopsin

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Radiative emission has been observed from the retinylidene chromophore of bacteriorhodopsin, the purple membrane protein of Halobacterium halobium.

LIGHT emission has never been observed from the retinylidene chromophore of any rhodopsin[1]. In addition, no vibronic structure has been detected in the absorption spectrum of the chromophore of rhodopsin or of any isomer of retinal. Furthermore, no reproducible electron paramagnetic resonance signal has been recorded from any rhodopsin. These are the principal reasons why little is known about the electronic structure of the retinylidene chromophore of rhodopsin.

Theoretical calculations[2] and studies of model polyenes[3,4] have been the principal approaches used to try to understand the electronic structure of various rhodopsins. We report here the first observation of light emission attributable to the retinylidene chromophore of a rhodopsin. This study and several other investigations in progress in our laboratory are directed toward resolving the excited state ordering in the retinylidene chromophore and understanding changes in these excited states after a photon interacts with rhodopsin.

Emission spectroscopy of rhodopsin

While applying resonance Raman spectroscopy to study the ground state vibrational structure of the retinylidene chromophore of several rhodopsins and their intermediates[5–10], we

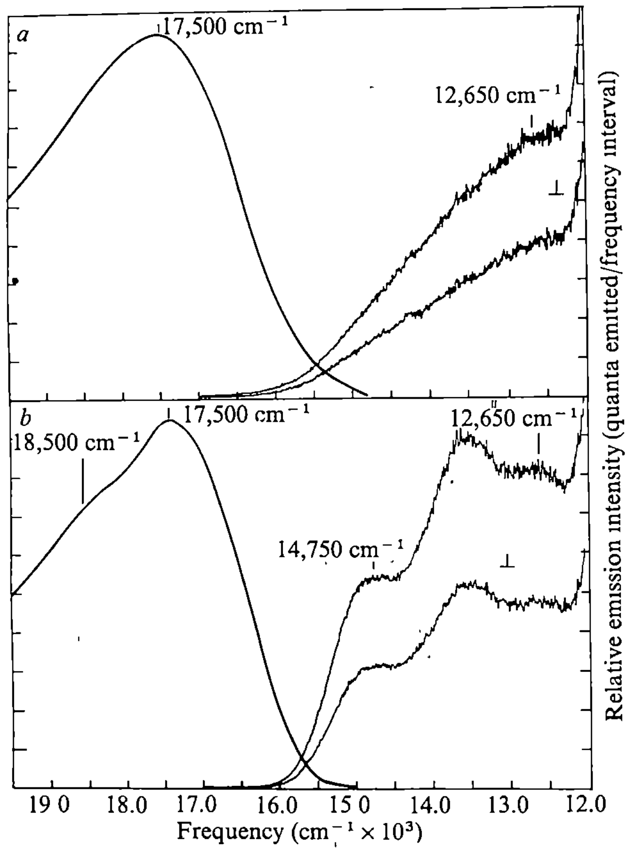


Fig. 1 *a*, Room temperature relative absorbance in arbitrary units scaled from ref. 21 and room temperature relative emission intensity, corrected for instrument response in units of number of quanta emitted per unit frequency interval. The ordinate scale is in thousands of wave numbers. The excitation source for the emission is the 514.5-nm line of an argon ion laser and intensities both parallel and perpendicular to the incident laser polarisation are shown. The apparent rapid rise in the extreme low energy portion of the corrected spectra is artificial and due to division of a nearly constant dark count for the photomultiplier by the vanishing instrument response function. *b*, Low temperature (77 K) relative absorbance in arbitrary units scaled from ref. 21 and 77 K emission intensity corrected for instrument response in units of number of quanta emitted per unit frequency interval. The ordinate scale is in thousands of wavenumbers. The excitation source for the emission is the 580.0-nm line of a CW rhodamine 6G dye laser Intensities both parallel and perpendicular to the incident laser polarisation are shown.

have developed, as a by-product, a spectrometer which is also a sensitive fluorimeter The excitation source for our spectrometer is a tunable dye laser, which provides an ideal source for the stimulation of weak emissions.

We have used our Raman spectrometer to detect emission from rhodopsins and have observed re-emission from bacteriorhodopsin. Bacteriorhodopsin has been studied by Stoeckenius *et al.*[11–14], who have shown that *Halobacterium halobium* uses this rhodopsin to convert light energy into chemical energy. Bacteriorhodopsin has an absorption maximum at 570 nm, and when the retinylidene chromophore is extracted from this species it has an all-*trans* conformation[15]. Bacteriorhodopsin occurs in purple membrane patches in the plasma membrane of the bacteria. These patches can be isolated as membrane fragments which contain the rhodopsin in a crystalline hexagonal array, as shown by diffraction experiments[12,16] The array of rhodopsins is in clusters of three in the membrane with a threefold axis of symmetry perpendicular to the plane of the membrane at the centre of each cluster[16]. We have used bacteriorhodopsin prepared by the procedure of Oesterhelt and Stoeckenius[17]. The membrane fragments were suspended in water at room temperature and in a glycerol–water glass at 77 K. Similar results were obtained from membrane fragments isolated from an S-9 mutant of the bacteria, which is believed not to contain carotenoids.

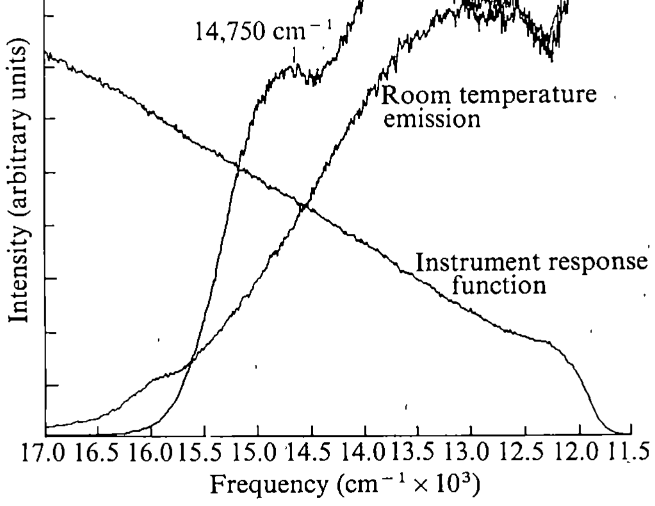
All the spectral data shown in Figs 1–3 were obtained on a laser Raman spectrometer using a red sensitive RCA C31034 photomultiplier with photon counting electronics. Each data point was scaled digitally with the measured instrument response function as determined using a quartz–halogen standard lamp. The instrument response function can be seen in Fig. 2 superimposed on the room temperature and 77 K corrected spectra. The excitation wavelengths used for these measurements were obtained from either an argon ion laser or a continuous wave rhodamine 6G tunable dye laser. All absorption spectra were recorded on a Cary 14 spectrophotometer.

Quantum efficiency

Bacteriorhodopsin is ideal for the study of re-emission from rhodopsins because the membrane fragments contain only one protein[12,16], which is the rhodopsin. The emission maximum we observed at room temperature (Fig. 1*a*) occurs in the near infrared region of the spectrum and is shifted by ~ 4,850 cm^{-1} from the room temperature absorption maximum. There is minimal overlap between the absorption and emission spectra at room temperature and at 77 K. The room temperature quantum efficiency was determined to be between 1.2 (10^{-4}) and 2.5 (10^{-4}), using reduced photosynthetic reaction centres from the bacterium *Rhodopseudomonas spheroides* as a standard, according to the method of Parker and Rees[18]. A quantum efficiency of 1.0 (10^{-3}) was assumed for the reaction centres[19].

Reaction centres are an ideal standard for this study because of their relatively low emission quantum yield and because of the high degree of spectral overlap with the emission of bacteriorhodopsin. Absorbance spectra, taken before and after the measurement of reaction centre emission, indicated no changes in the preparation. The absorbance of bacteriorhodopsin used for the quantum yield was 0.687, while the reaction centres had an absorbance of 0.585. The excitation source for just the quantum efficiency measurements was a quartz–halogen lamp in combination with a Corning 4-96 filter and a broad band 550-nm interference filter. The instrument response function was determined with a lamp having a

Fig. 2 Relative instrument response superimposed on the room temperature and 77 K corrected emission spectra. The emission spectra are in units of number of quanta emitted per unit frequency interval The band observed at approximately 16,000 cm^{-1} in the room temperature emission spectrum is due to the ~3,400-cm^{-1} band in the Raman spectrum of H_2O.



colour temperature of 2,860 K. The spectra were recorded in an analogue mode with a photomultiplier having an S-1 response and were subsequently digitised to allow for a point-by-point subtraction of background due to scatter. Scattering spectra were obtained using a Ludox solution. Furthermore, the spectra were scaled by the instrument response function and corrected to give the integrated area in terms of photons emitted per incident photon. No effect on emission intensity was observed due to deaeration. A detailed study of the quantum efficiency in various conditions will be reported elsewhere.

Polarisation of the emission

The emission intensities of the components parallel and perpendicular to the incident laser polarisation were determined using a Pockels cell to rotate the laser polarisation with respect to a fixed analyser. We recorded the parallel and perpendicular emission components in alternate channels at each frequency setting of the monochromator. The data were separated, scaled and plotted using a digital computer and are presented in Fig. 1*a* and *b*. The emission polarisation$[(I_{||}-I_{\perp})\,(I_{||}+I_{\perp})]$ which we have measured in a 90° scattering geometry varies from 0.23±0.02 to 0.49±0.02 at both room temperature and 77 K when excitation frequencies of 19,436 cm^{-1} and 17,241 cm^{-1} are used. Such variations in the degree of polarisation have been observed for pigments in *Chlorella*, for example[20]. These variations when different experiments are compared result from varying amounts of partial orientation in the gravitational field of the suspension of membrane fragments containing the well-ordered pigment molecules. Because of this effect, one way to determine the degree of emission polarisation accurately is to measure it in the presence of a magnetic field: this is in progress in our laboratory. It is interesting, however, that the degree of polarisation seen in Fig. 1*a* and *b* remains constant throughout the observed emission band within the time scale of any one measurement. Thus these data suggest that the emission emanates from the excited state of a single species.

This conclusion is supported by a plot of the ratio of the intensities of the components at 12,650 cm^{-1}, 13,650 cm^{-1} and 14,750 cm^{-1} in the 77 K spectrum as a function of exciting laser frequency. The data plotted in Fig. 3 are for the exciting laser frequencies which produce the largest changes in the concentration of the photoproduct (unpublished results of R. Lozier and W. Stoeckenius). (The photoproduct at 77 K is a red-shifted intermediate with an absorption maximum ~ 635 nm; refs. 21 and 22). Therefore these experiments also indicate that the components observed in the 77 K spectrum arise from a single species, and this suggests that the components are indeed vibronic fine structure on the emission. The spacing between the components is ~ 1,000 cm^{-1} and ~ 1,100 cm^{-1} in the emission spectrum, and a shoulder is detected at a similar spacing on the high energy side of the 77 K absorption maximum (Fig. 1*b*).

Assignment of observed emission

Light induces photochemical changes in bacteriorhodopsin similar to those observed in all rhodopsins. This photochemical behaviour changes the concentration of rhodopsin with different actinic wavelengths. A two-laser-beam technique can be used to control the photochemical behaviour while observing the emission spectrum. This is particularly feasible at 77 K, where only two components are present, bacteriorhodopsin and the batho form absorbing at ~ 635 nm (refs 22 and 23). These two components are photoreversible at 77 K and exist in a photostationary equilibrium depending on actinic wavelength (unpublished results of R. Lozier and W. Stoeckenius). We have obtained data by the use of two actinic wavelengths. The data are summarised in Table 1. The principal feature of these experiments is similar to the enhancement effects observed in photosynthetic systems, where the effect of two simultaneous actinic irradiations is not equal to the sum of the individual effects[24,25]. The sum of the emission intensities stimulated by 10 mW of illumination at 636.0 nm and by 1 mW of illumination at 514.5 nm obtained in separate experiments is less than the emission observed when the sample is irradiated simultaneously with both beams. In addition, the sum of emissions stimulated with 1 mW of 636.0 nm and 10 mW of 514.5 nm is less than the emission obtained in a single dual beam experiment with the same laser powers and wavelengths. The differences we observed can be explained in terms of changes in the photostationary concentrations of bacteriorhodopsin and the photochemically produced batho form. This is independent of any assumption about the wavelength dependence of the quantum efficiency of the emission and the photochemical quantum yield. The reason for this is that, in the experiments tabulated in Table 1, only the laser powers and not the wavelengths have been changed.

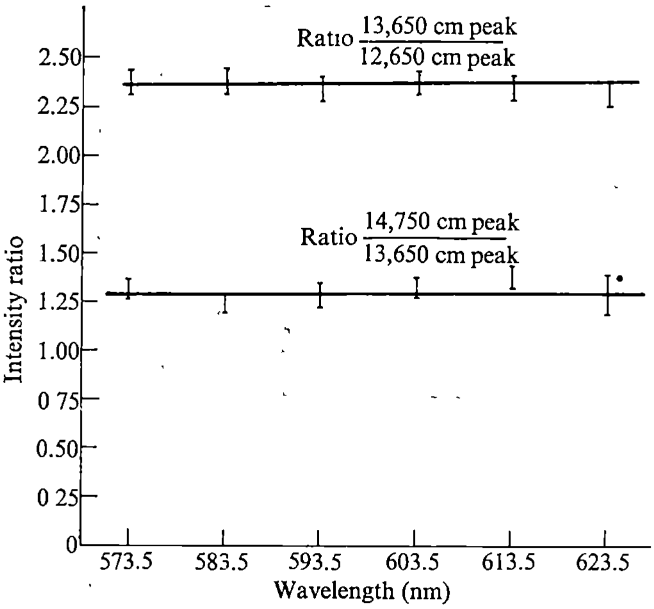


Fig. 3 Ratios of intensities of the bands observed at 14,750 cm^{-1}, 12,650 cm^{-1} and 13,650 cm^{-1} against wavelength. The excitation source is a continuous wave rhodamine 6G dye laser. The ratios shown are calculated from unscaled data

Table 1 is consistent with the interpretation that the primary emitter, in the region we detected the emission spectrum, is bacteriorhodopsin. For example, the addition of 1 mW at 636.0 nm in experiment 6 not only adds its own emission intensity but also increases the steady state concentration of bacteriorhodopsin to a value which is greater than that of experiment 5. Thus the intensity observed in experiment 6 is greater than the sum of the intensities observed in experiments 4 and 5. A similar explanation can be made for experiments 1, 2 and 3.

A comparison of the room temperature and 77 K emission

Table 1 Emission intensities observed using a dual beam technique

Experiment	Laser I (636.0 nm) (mW)	Laser II (514.5 nm) (mW)	Emission intensity proportional to (quanta emitted/incident quanta)	
1	10	0	267	→ 336
2	0	1	69	
3	10	1	386	
4	1	0	26	→ 720
5	0	10	694	
6	1	10	738	

All dual beam experiments make use of both an argon ion laser to produce radiation at 514.5 nm and a CW rhodamine 6G dye laser for 636.0-nm irradiation. The emission intensities have been corrected so that they are given in relative number of quanta emitted per number of incident quanta. Each emission intensity is accurate to ±2%.

spectra (Fig. 3) is also consistent with the suggestion that bacteriorhodopsin is the primary emitter. It is known that, based on the kinetic constant at room temperature for the decay of the batho intermediate to its thermal product[22,23], appreciable concentrations of this intermediate cannot be present at room temperature. Conversely, at the temperature of liquid nitrogen a significant fraction of the photostationary mixture is in the batho form. When this is considered together with the similarities in the room temperature and 77 K spectra, with the observation that the ratio of the vibronic components remains constant at various exciting wavelengths (Fig. 3) and with the results of the two beam experiments, we conclude that the emission emanates from bacteriorhodopsin and that the batho form does not emit in this region of the spectrum. This conclusion is supported by excitation spectra which we have obtained at room temperature.

The excited state

Our experiments indicate that an excited state of bacteriorhodopsin is responsible for the emission spectra we observed. Based on the integrate strength of the absorption for the allowed transition, a radiative lifetime for emission from the ^1B_u state would be 7 ns But measurements of the lifetime by the use of a mode-locked tunable dye laser and by phase-modulation techniques suggest that the calculated intrinsic lifetime of the emission is between 0.1 and 1 μs (our unpublished results with M. Marcus, M Hirsch and A. Kriebel). Thus the emission cannot originate from the ^1B_u state. The observed variation in the lifetime of the emission could result from exciton interactions among the chromophores at high laser intensities.

There is, however, a separation between the absorption and emission bands (Fig 1*a* and *b*) similar to the separation observed by Hudson and Kohler[4] in the spectra of model polyenes, the radiative lifetimes we observe are comparable with those detected by Hudson and Kohler[26] for model polyenes. The data on these model systems have been taken to mean that the lowest energy excited state in these polyenes is a ^1A_g state. The occurrence of such a perfect ^1A_g state, or a perfect ^1B_u allowed state, in the retinylidene chromophore is unlikely especially in view of the large excited state dipoles that are measured (personal communication from R. Mathies and L. Stryer) and the observation of a circular dichroism spectrum in bacteriorhodopsin[27]. Thus our data can only be interpreted to mean that the emission emanates from some state other than the allowed state into which light energy is absorbed. Picosecond absorption measurements have suggested the existence of a transient intermediate produced before the batho intermediate which occurs in < 6 ps (ref. 28). This transient could represent the absorption of light by the state from which we are observing emission.

We can deduce from our data some information on the character of the excited state from which emission is observed. For example, the observation of vibronic structure in the 77 K emission spectrum suggests that the excited state involved in the emission has electron density delocalised in the polyene. This would increase the bond order of single bonds and decrease the bond order of double bonds. It has been suggested[2] that a lack of vibronic structure in the absorption spectrum can be attributed to the sterically hindered cyclohexene ring in the retinylidene chromophore Thus the C6–C7 single bond between the cyclohexene ring and the isoprenoid chain has a very broad ground-state potential. But if in the excited state the electron density is delocalised, the bond order of the C6–C7 single bond should increase and this in turn should narrow considerably the excited state potential surface for this torsional angle coordinate. Such an effect would reduce the conformational freedom of the cyclohexene ring relative to the isoprenoid chain and this should result in the vibronic structure observed in our emission spectrum. We believe it is significant that resonance Raman experiments on the batho intermediate (our unpublished results) which is stabilised from the excited state also indicate (from the frequencies of the C–CH$_3$, –C=N– and C=C vibrations) significant reduction in the C=C bond order and charge polarisation in this first stable product which results from a photon interacting with bacteriorhodopsin. Thus when the fluorescence and resonance Raman data are analysed together the excited state structure and electronic distribution seem to be a precursor and initiator of the stable batho photochemical product.

The blueshift we observed in the emission spectra when changing from room temperature to 77 K suggests that the chromophore is surrounded in the lipoprotein matrix by dipolar groups which have not relaxed fully at low temperature. In addition it is clear from the two beam experiments completed at 77 K that the batho form does not have the same excited state as this rhodopsin. Some theories of the photochemistry of rhodopsin have predicted that the excited states of rhodopsin and the batho intermediate are identical. Finally, the fact that no emission is observed for the batho form in the region of the spectrum where our apparatus is sensitive strongly suggests that the photochemical act which stores light energy in the 635-nm intermediate quenches radiative emission.

In conclusion, we feel that these observations of emission from a rhodopsin open new opportunities to understand the excited state ordering and structure of the retinylidene chromophore. Furthermore, these emission spectra will be important in understanding the primary photonic event which produces a red shift in the chromophores of all rhodopsins. Thus these data on the initial photochemically induced changes in the retinylidene chromophore should yield new insights into the conversion of light energy into chemical energy by rhodopsin, and should have an important role in the formulation of a mechanism of energy transduction by rhodopsin.

We thank Professor Walther Stoeckenius for bacteriorhodopsin and helpful discussions. We also thank Dr Richard Lozier for stimulating discussions; Professors A. Albrecht and H Mahr, Dr A. Kriebel, and M. Hirsch for obtaining the emission lifetime, and Professor R. Clayton for encouragement and obtaining the room temperature quantum efficiency A L. is an Alfred P. Sloan fellow; J.P.S. held an Eastman Kodak graduate fellowship; G.J.P. was supported by a graduate fellowship from the Ford Foundation. This work was supported by a grant from the National Institutes of Health.

Received August 11, 1975, accepted February 27, 1976

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Bicycle-pedal model for the first step in the vision process

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*Computer simulation of the molecular dynamics of retinal during its photoisomerisation inside a restrictive active site gives a detailed model for the sequence of events in the first step of the vision process. It is proposed that the prelumirhodopsin intermediate contains a strained all-*trans *retinal molecule produced directly and rapidly from the 11-cis, 12-s-*trans *conformation in rhodopsin by a bicycle-pedal isomerisation. The model reproduces the main experimental observations and explains how the protein makes the photoisomerisation path unique.*

IN the first step of the process of vision the rhodopsin molecule (a complex of 11-*cis* retinal and the protein opsin) absorbs a photon and undergoes a unique transition to the prelumirhodopsin intermediate[1]. (For a review of recent work see refs. 2 and 3.) Some of the most definite information about this reaction emerged from picosecond laser[4] and resonance Raman[5,6] experiments as well as from studies of artificial pigments[7-9]. The transition from rhodopsin to prelumirhodopsin takes less than 6 ps (6×10^{-12} s) and the retinal moiety in this intermediate is characterised by strong resonance Raman lines that do not appear in rhodopsin[5]. The interaction with opsin directs the pathways of photoisomerisation of quite different retinal isomers to the same all-*trans* final form[8]. In spite of the potential usefulness of this information there is no unique interpretation for any of these findings and the nature of the first step remains unresolved. It is reasonable to assume that the rhodopsin molecule uses the light energy for the photoisomerisation reaction of 11-*cis* retinal as fast as possible or else the excited state will be deactivated by radiation and radiationless processes. It is not clear, however, how the photoisomerisation can be accomplished in as large a molecule as retinal in such a short time[4,5] and how it can be done at all in a restricted active site[10]. Furthermore, there is no clue to the most intriguing mechanism by which the protein leads to only one final isomer from all the different initial complexes[8].

Recent theoretical developments[11-15] make it possible to analyse the available experimental information and to calculate reliably ground and excited state equilibrium geometries[12,13], absorption spectra[12,13] and resonance Raman spectra of retinal and related molecules (ref. 14 and my unpublished work with M. Karplus). It is also possible to simulate the dynamics of the photoisomerisation process and to monitor the conformational changes as a function of time[15]. In addition the interaction between the chromophore and hypothetical charges on the protein can also be evaluated[16]. The available theoretical approaches can be used to study various models for the visual excitation.

Here I propose a detailed model for the molecular events after the absorption of a photon by rhodopsin. The recently developed theoretical methods[12-16] are used to test the model against the principal experimental facts about the first step of the vision process and to stimulate the molecular dynamics of retinal during photoisomerisation. The following general assumptions are introduced in this model.

(1) The retinal is bound to a restrictive active site in which the cyclohexene ring, at one end of the molecule, is trapped in a hydrophobic cleft (this suggestion is supported by binding experiments[9] and by analogy to other enzymes) and the other end of the molecule (the "tail end") is bonded by a Schiff base bond (C=N) to a lysine residue of the protein. (2) No major conformational changes of the protein are involved in the first step (for discussion see refs 2 and 3). (3) The light energy must be used for isomerisation around the *cis* double bond, since this is the main energetic barrier of the visual process and it would be rather difficult to perform fast thermal isomerisation after the dissipation of the light energy. (4) In addition to these assumptions which are the working hypotheses of the model it is also assumed that the chromophore in rhodopsin is set initially to the 11-*cis*, 12-s-*trans* conformation (this assumption is supported by ref. 7 as well as by the work reported here) and that the Schiff base nitrogen is protonated[5,6]. It is further assumed that the protonated Schiff base bond is protected from hydrolysis by a negative group of the protein (a salt bridge) which increases the basicity of the Schiff base nitrogen.

Main features of the model

In the proposed model, described schematically in Figs 1 and 2, the chromophore is placed in the protein active site which is defined by assumptions (1) and (4). After absorption of light the *cis* bond is propagated to the tail end of the retinal chain by a 'bicycle-pedal'-like motion (see below). In this motion the molecule first changes its conformation by moving downhill on the potential surface of the excited singlet state and then crosses to the ground state once the C11–C12 torsional angle is approximately 90°. In the ground state the molecule becomes trapped in a strained all-*trans* (Fig. 2*d*) form, where the bond to the lysine residue forces the chain to bend and the salt bridge is stretched relative to its equilibrium distance in rhodopsin. This strained all-*trans* is the prelumirhodopsin intermediate in which the Schiff base nitrogen is less basic than in rhodopsin since its salt-bridge length is larger.

An important feature of the model is that all the isomerisation modes of different possible pigments can be executed by a concerted rotation about parallel pairs of double bonds ('bicycle-pedal motion'), as described in Fig. 2. In particular, the *cis* conformation can propagate from different double bonds to the terminal C=N bond. Thus, the protein makes the retinal change its conformation along a one-dimensional reaction path (essential for the large photon efficiency) simply by constraining the ends of the molecule so that it must move in a 'bicycle-pedal' motion rather than in all the possible modes of motion which are possible in solution[8].

Detailed examination of potential surface

The retinal intramolecular energy surface and its analytical derivatives were evaluated by the method already developed[12,13]. Since the barriers for rotation around double bonds are of special importance here, we recalibrated the potential parameters with particular emphasis on the reproduction of the

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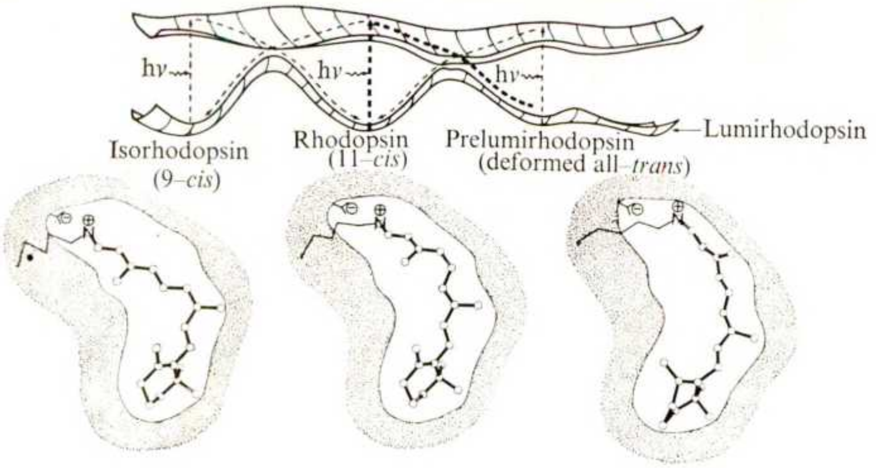

Fig. 1 A schematic description of the proposed model for the first step in the vision process. The figure presents the variation of the ground and excited state potential surfaces as a function of the adiabatic reaction coordinate and an effective perpendicular coordinate which represents all other coordinates. The change of the chromophore conformation in the enzyme active site are presented schematically below the surfaces. The thick broken line describes the primary visual process when the retinal chromophore of rhodopsin is excited by light to the first excited singlet, slides down, crosses to the ground state and becomes trapped in the prelumirhodopsin conformation. The thin broken line describes the mechanism of reversible transition between different intermediates.

potential surfaces of the ground and the first excited singlet and triplet states of ethylene. The calculation includes a configuration interaction treatment of all the single excitations and the low lying double excitations. The parameters for the Schiff base nitrogen were determined by an extensive fit to many independent properties of nitrogen-containing compounds (my unpublished work with A. Lappicesela)[12]. The σ electrons' charge distribution in the protonated Schiff base was determined by an all-valence electron approach[16]. In conventional semi-empirical quantum mechanical calculations the energy of the first excited singlet does not decrease smoothly as the C11–C12 double bond is twisted from 0° to 90° giving an activation barrier so that the singlet photoisomerisation becomes quite inefficient. We have found, however, that whenever the calculations include double configuration interaction treatment and Lowdin overlap correction[12,13] the energy decreases monotonically on twisting to 90° without any activation barrier.

The effect of the active site was included in the calculation by taking into account the main features of steric restriction and electrostatic interaction. The steric restriction on both the ring and the tail was simulated by forcing the ring carbons and the α carbon of the lysine residue to stay close to their positions in 11-*cis*, 12-s-*trans* retinal (the starting conformation). The electrostatic interaction with the enzyme was simulated by the recently developed "microscopic" dielectric model[16], using the atomic polarisability and average density of a typical protein (in this case lysozyme). It was found that while the effect of negative charge at various points along the chain can account for the spectral shifts between different pigments, its effect in modifying the surfaces is much smaller than that which could be expected in view of the sudden redistribution of the charges of the isolated chromophore on twisting of the C11–C12 bond to 90° (ref. 23). This effect is discussed below. Thus although the presence of external charges can reduce the spacing between the ground and excited states in the 90° region, the molecular dynamics calculation was performed with only a single negative charge near the N^{ε} of the lysine residue.

Simulation of photoisomerisation dynamics

If the transition between rhodopsin and prelumirhodopsin involves the proposed *cis–trans* photoisomerisation, then any simulation of this process must reproduce the fact that the transition is faster than 6 ps. The simulation should also account for the large quantum efficiency of the reaction and for the concept of a complete isomerisation in a restricted active site. Here the "semiclassical trajectory approach" is used to investigate surface crossing in photoisomerisation[15]. In this approach one evaluates classically the intramolecular motion (trajectory) on the excited state surface and then uses the calculated variation of the coordinates with time to evaluate quantum mechanically the transition probability to the ground state. At each point of large transition probability the trajectory is split into two parts—one crosses to the ground state and the second continues on the excited state surface. Each of these trajectories is weighted by the corresponding transition probability. Here we are dealing with a strong singlet–singlet coupling and the adiabatic representation[17,18] rather than the diabatic[15] one is used. The result of a typical trajectory is presented in Figs. 3 and 4, which show that the molecule can perform isomerisation inside a restrictive active site in about 2×10^{-13} s. This fast isomerisation is due to the small moment of inertia of the bicycle-pedal motion. Figures 3 and 4 also show that the molecules cross to the ground state with 30% probability during a single pass through the 90° region. Thus after about three passes (which corresponds to about 6×10^{-13} s) the probability of the molecule being in the ground state is almost 100%. The calculation clearly shows that a complete

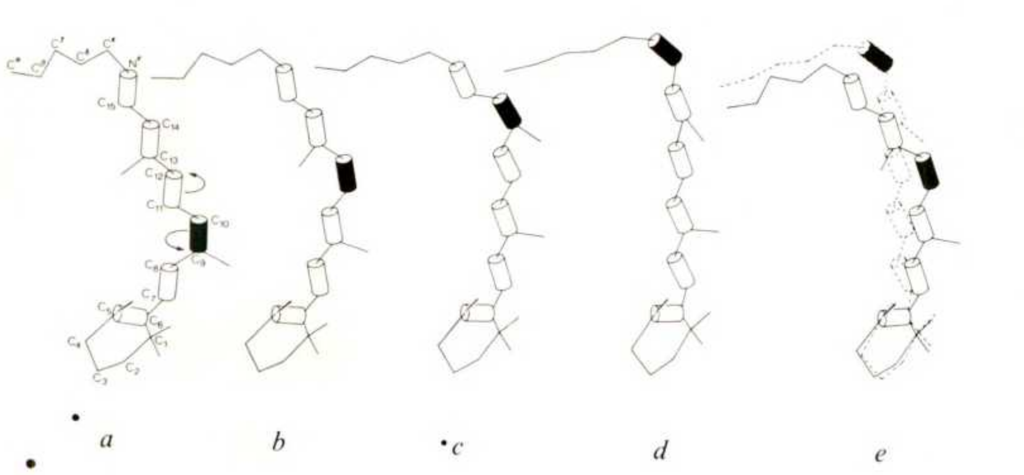

Fig. 2 Typical elements of the 'bicycle-pedal' mechanism. *a–d*, 9-*cis*, 11-*cis*, 13-*cis* and 15-*cis* isomers, respectively; *e*, the one-step concerted rotation about the 11–12 and 15–16 bonds which is the proposed motion in the visual excitation. Clearly with a system of parallel double bonds the *cis* bond can be transferred to any point without large shift into the atomic coordinates.

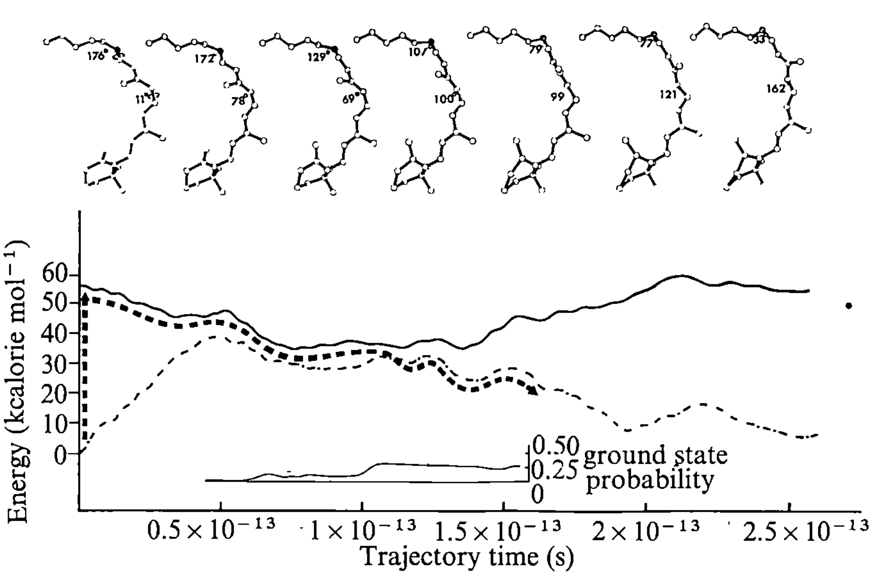

Fig. 3 Computer simulation of the first step in the vision process The figure presents a single trajectory which begins on the first excited singlet and crosses to the ground state at the point of maximum transition probability ($\varphi_{11-12} = 95°$) The potential energy of the ground and the first excited singlet are described by the broken and solid lines, respectively, as a function of the trajectory time while the conformational changes along the trajectory are presented above with the corresponding values of φ_{11-12} and φ_{15-16} The trajectory was obtained in the complete Cartesian space starting with the ground state equilibrium coordinates and zero kinetic energy The ring and the tail ends were constrained by assigning masses of 1,000 atomic units to the C1 and C5 ring carbons and to the Cα of the lysine residue. The humps on the potential surface along the trajectory are due to transfer of kinetic energy to potential energy in several bond-stretching, angle-bending and torsion vibrations The trajectory describes a dynamical rather than an equilibrium process and the dissipation of the excess torsional potential energy in the many normal modes (~200) of the system is not complete Thus in some energy fluctuations much of the kinetic energy is converted back to potential energy The probability of being at the ground state $a_0{}^2(\tau)$ is given as a function of the trajectory time as:

$$a_0(\tau) = \int_0^\tau \left[\langle \Psi_0 \mid \frac{\partial \Psi_1}{\partial t} \rangle a_1(t) \exp\left\{ -(i/\hbar) \int_0^t (E_1 - E_0) \mathrm{d}t' \right\} \right] \mathrm{d}t$$

where Ψ_0 and Ψ_1 are the ground and excited state electronic wave functions and E_0 and E_1 are the corresponding potential energies. In the actual evaluation of the *cis–trans* quantum yield the trajectory is split first at $\varphi_{11-12} = 82°$ where a trajectory with ~0 1 probability crosses to the *cis* ground state while the main trajectory continues in the excited state up to the point $\varphi_{11-12}=95°$ where it splits again with ~0 2 crossing to the ground state (this trajectory is shown in Fig 4), while another trajectory proceeds on the excited state with 0.7 probability This splitting procedure continues until the probability of the excited state trajectory is less than 0.1.

cis–trans photoisomerisation can be accomplished in less than 6 ps in contrast to previous suggestions[4]. Another important point is the fact that the calculated quantum efficiency is larger than 50%, indicating that the model system is almost as efficient as rhodopsin.

In contrast to the 'bicycle-pedal' motion the conventional photoisomerisation path involves a large rotation about the *cis* bond of either the ring[10] or the tail end. The resulting motion will be very slow due to the large moment of inertia and high probability for collision with the walls of the active site.

Resonance Raman and absorption spectra

The resonance Raman (RR) technique can provide unique information about the visual pigment as it measures the vibrational spectrum of the retinal inside the active sites of the protein Recent measurements have shown that prelumirhodopsin is characterised by lines in the 850–950 cm^{-1} region[5], which are not observed in rhodopsin or in Schiff base of retinal in solution. Our method for evaluation of vibronic transition intensities[14] can be used for direct calculation of the RR spectrum. This approach reproduces the main features of the RR spectra of Schiff bases of retinal in solution[14]. Model calculations have shown that the special lines of prelumirhodopsin can be reproduced if the chromophore is strained (my unpublished results with M. Karplus). The calculation of the RR spectrum of the present model of prelumirhodopsin (Fig. 2*d*) also reproduces two very strong lines at the 900 cm^{-1} region. These are due to a relatively large change in the geometry of the strained chromophore on electronic excitation.

Previous calculations of the dependence of the absorption maximum (λ_{max}) on external charges are reviewed in refs 2 and 3. In contrast to these works I include in my calculations the major effect of the protein polarisability[16]. This enables me to analyse properly the effect of external charges on λ_{max}. The degree of protonation was explicitly taken into account by calculating the σ charges of the retinal, the proton and the counter ion by an all-valence electron approach. I found that the redshift between rhodopsin (498 nm) and a protonated Schiff base of retinal in solutions (440–480 nm) can be accounted for by a calculated redshift of λ_{max} from 460 to 485 nm which is obtained when the salt bridge is placed in a more polar environment than the rest of the chromophore. An additional redshift of about 30 nm is obtained when the salt bridge is stretched from 2.5 to 4.5 Å. A further redshift of as much as 40 nm is obtained by another charged group properly placed near the chain, whereas a redshift of about 20 nm is obtained by ~ 10° twist about a double bond (obviously the shift can be increased by a larger twisting). Thus a significant part of the redshift between prelumirhodopsin (543 nm) and rhodopsin can be accounted for by a stretching of the salt bridge. The possibility, however, cannot be excluded that the redshift is due to the effect of double bonds twisting and/or changes in the distances between the chain and some negative or positive group. The inclusion of the polarisability reduces by about half the previously assumed effect of external charges[2,3]. Thus, it is likely that some part of the redshift between prelumirhodopsin and rhodopsin is due to twisting of double bonds. Essentially, Figs 1 and 3 show that a redshift of any magnitude can be obtained depending on the equilibrium value of φ_{11-12}, which is determined by the balance between the intramolecular potential of the chromophore and the constraint of the protein.

Another related fact, which introduces difficulties in other models[19], is the finding that the transition dipole is almost parallel in rhodopsin and the different bleaching intermediates[25]. This is clearly reproduced by my model which keeps all the double bonds parallel during the different stages of visual excitation.

Quantum efficiency and reversibility

An interesting and so far unresolved problem concerns the facts that prelumirhodopsin is a common intermediate for both rhodopsin and isorhodopsin I (9-*cis*)[5,10] and that the quantum efficiency of rhodopsin is about 0.7 while that of both isorhodopsin I and isorhodopsin II (9,13-*dicis*) is about 0.3 (ref. 8). This behaviour can also be studied by the semiclassical trajectory approach[15]. The calculated quantum efficiency for my rhodopsin model is about 0.6 A preliminary calculation starting with isorhodopsin I gave about 0 3 probability for

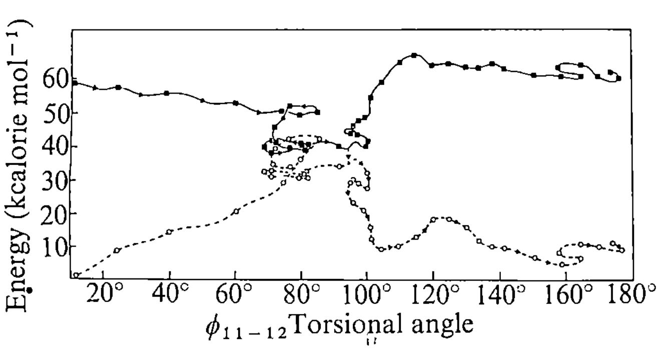

Fig. 4 The relationship between the potential surfaces and the φ_{11-12} torsional angle along the sample trajectory which is presented in Fig. 3 The trajectory time intervals (6×10^{-4} s) are represented by (○) and (■) for the ground and excited state, respectively. The actual potential surface taken by the trajectory is designated by (—›). The figure shows that the molecule forms some 'activated complex' and stays a relatively long time in the 90° region. The different values of the potential energy for similar values of φ_{11-12} are due to changes in other coordinates. Thus after the surface crossing φ_{11-12} varies slowly and the changes in the potential are due to changes in φ_{15-16} and φ_{9-10} as well as in various bond lengths and bond angles.

photoisomerisation in a 'bicycle-pedal' motion to rhodopsin (a similar part proceeds directly to prelumirhodopsin in a concerted rotation about the 9–10 and 15–16 bonds). With a second photon the rhodopsin proceeds with 0.7 probability to prelumirhodopsin. This suggestion can account for the fact that isorhodopsin I is about half as efficient as rhodopsin. The isomerisation of the isorhodopsin II (9,13-*dicis*) can be explained as described in Fig. 5: the molecule can move directly to prelumirhodopsin by a concerted bicycle-pedal rotation about the 9–10 and 13–14 bonds Alternatively the molecule can change its conformation to 11,13-*dicis* and then directly to all-*trans* by a second 'bicycle-pedal' motion of the two adjacent *cis* bonds.

The reversibility of photoisomerisation between prelumirhodopsin, rhodopsin and isorhodopsin (my unpublished results with M. Karplus) is accounted for by the fact that absorption of photons in one isomer populates its excited state, which can be relaxed to the ground states of the other isomers (Fig. 1).

Alternative explanations

Although other models of the visual excitation have been proposed[1,10,19-24], they did not provide unique isomerisation paths nor a detailed proposal for the conformation of prelumirhodopsin. Nevertheless, these models have some interesting features which are worth considering in terms of alternative explanations for the observations.

Recent calculations[23] have shown that the excited state charge distribution of the chromophore might change drastically on twisting of the C11–12 bond to 90°. A "short-lived electrical signal" mechanism was proposed as the primary event in the vision process. Such a mechanism seems to be unlikely as long as it implies a very short pulse rather than an irreversible change. The strong dependence of the excited state polarisation on the C11–12 torsional angle, however, presents the intriguing possibility that prelumirhodopsin has a twisted double bond where an external charge stabilises the excited singlet so that it becomes a ground state. If such a mechanism were operative along the 'bicycle-pedal' pathway it could account for many observations. My calculation, however, never reproduced this stabilisation effect, even with drastic changes of the semi-empirical parameters: that is, if the retinal charge distribution is not allowed to change in response to any external charge effect, then an optimally placed negative group could push the twisted excited state as much as 50 kcalorie mol^{-1} below the twisted ground state. When, however, a complete charge redistribution was allowed in my calculations, I was unable to push the excited state more than 5 kcalorie mol^{-1} below the ground state. This is because the presence of the negative charge near various points with a positive charge in the excited state of the isolated molecule induces positive charges in the ground state at the same points, which in turn makes the excited state charge less positive. I feel, however, that my model calculations cannot exclude completely the possibility that the chromophore in prelumirhodopsin is trapped in a twisted form by a strong interaction with some charged group of the protein.

Another suggestion is that the protonated Schiff base releases a proton in the primary event in the vision process[24]. The problem with this model is that the photoisomerisation must then be accomplished by thermal energy (this would cost about 25 kcalorie mol^{-1}) and the light energy will be wasted. Furthermore, the proton dissociation will cause a blueshift rather than a redshift in the absorption spectrum of prelumirhodopsin.

The possibility that the photoisomerisation is accomplished through the triplet state rather than the singlet state cannot be excluded completely. Recent experiments[26], however, support the singlet mechanism, and my study has shown that there is a significant barrier for isomerisation in the triplet. An estimate of the triplet–singlet coupling indicates that this coupling gives less efficient surface crossing than the non-adiabatic singlet–singlet coupling.

We cannot disregard completely the possibility that the prelumirhodopsin intermediate is not in a strained all-*trans* conformation but in other conformations along the reaction path between 9-*cis* and 11-*cis*. Such a ground state conformation which requires a large twist about the 10–11 single bond as well as about the 9–10 and 11–12 double bonds might be possible if rhodopsin actually binds retinal in the 11-*cis*, 12-s-*cis*-form rather than 11-*cis*, 12-s-*trans* form. The photon efficiency of this system, however, should be less than 0.3 since the sum of the probabilities of isomerisation to 9-*cis* and of return to 11-*cis* is larger than 0.7.

It is clearly possible that the chromophore in prelumirhodopsin is in a much more twisted form than the one obtained by my potential surfaces. That is, the protein can provide such an external constraint which will stop the isomerisation in an earlier stage where φ_{11-12} and other torsional angles are strongly twisted. Obviously in this case the strain energy at prelumirhodopsin will be higher than that implied by the present calculation.

Implications of the model

The model presented here accounts for the main observations concerning the first step in the vision process. It is consistent with the ultra-fast transition from rhodopsin to prelumirhodopsin; the changes in the RR and absorption spectra during the transition, and the conservation of the direction of the transition moment. The model explains the uniqueness of the photoisomerisation process and its high quantum efficiency as well as the reversibility of transitions between the different pigments. The model is also consistent with present understanding of steric effects and charge stabilisation: that is, a

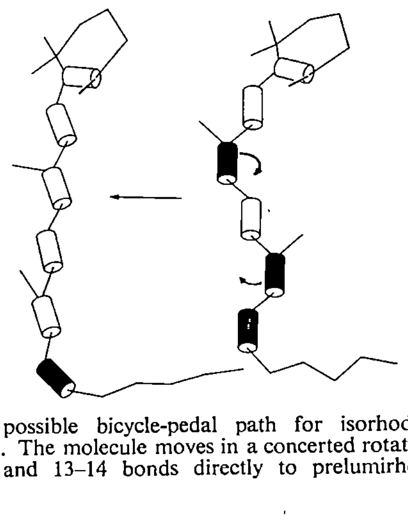

Fig. 5 A possible bicycle-pedal path for isorhodopsin II (9, 13 *dicis*). The molecule moves in a concerted rotation about the 9–10 and 13–14 bonds directly to prelumirhodopsin

steric strain by the protein is not effective if it can be relaxed by small displacements of the corresponding atoms. On the other hand, the steric effect may be important when the molecule is trapped in a twisted form by a constraint which can be relaxed only by large conformational changes. The charge effect (which was proposed as a very efficient way of controlling enzymatic reactions[16]) is used here with a salt bridge to the protonated Schiff base, whereas a fine tuning of the system can be obtained by other charged residues optimally placed along the chain.

It now seems that the first step of the vision process can be described as follows. Light absorption by rhodopsin populates the first excited electronic singlet state of the 11-*cis* retinal chromophore (which is bound at both ends as proposed here). The excited retinal slides in a very fast 'bicycle-pedal' motion to the 90° twisted singlet and then crosses to the ground electronic state while continuing to slide towards the *trans* form (Fig. 1). The protein defines a unique isomerisation valley for the conjugated chain by restricting the motion of both the ring end and the tail end and by some charge stabilisation. The use of the protonated Schiff base system with a proper charge stabilisation guarantees efficient singlet–singlet crossing and allows the system to perform very efficient isomerisation before other deactivation processes can take place After the surface crossing step, the chromophore is trapped in a high energy strained form (prelumirhodopsin) and the basicity of the Schiff base nitrogen is reduced as a result of the increase in distance between the negative residue and the N^{ϵ} relative to that in rhodopsin. In the next stage of the reaction (lumirhodopsin) λ_{max} is blueshifted by $\sim$45 nm relative to prelumirhodopsin. This shift might indicate changes in the polar environment of the Schiff base bond and/or other parts of the chromophore, as well as a decrease in the torsional deformation of some double bonds of the chromophore. Such changes are possible (when the temperature is increased and prelumirhodopsin is changed to lumirhodopsin) as a result of a partial release of the chromophore strain which involves some small conformational changes of the protein The release of strain and the new balance of the electrostatic interactions lead to more significant conformational changes of the protein during the steps lumirhodopsin to meta I to meta II. The spectral changes in the meta I to meta II step[1] indicate that the Schiff base proton is dissociated after further decrease in the basicity of the Schiff base nitrogen. In contrast to the situation in rhodopsin the non-protonated Schiff base in meta II can undergo hydrolysis by water. The protein conformational changes serve to convert the light signal into an electrical membrane current, somewhere along the pathway between prelumirhodopsin and meta II. These stages, however, do not involve further isomerisation of the chromophore.

It seems clear that in designing this photoconverter machine the two boundary conditions, which in turn establish the 'bicycle-pedal' mechanism, are important. In this way the system is forced to move in what is effectively one dimension along the complex energy surface as the *cis* bond is transferred backwards and forwards. Thus it seems that a promising way to simulate the surface crossing in rhodopsin might be achieved by a chemical approach of building a model compound with a Schiff base bridge between the ring to the end of the chain.

Yoshizawa and Wald have proposed[27] that the retinal molecule in this intermediate is in a strained all-*trans* conformation. Although the details of the transition between rhodopsin to prelumirhodopsin and the binding of the cyclohexene ring[1] were not clear at that stage, some features are common to both models.

I thank M. Levitt for encouragement and critical discussion and M. Karplus and B. Honig for stimulating discussions in the early stages of this work. This work was supported in part by the United States–Israel Binational Science Foundation and by the European Molecular Biology Organization.

Received January 7, accepted March 3, 1976

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The nucleus of Centaurus A

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The nucleus of Centaurus A contains a very compact radio component, and has recently been discovered to be a variable X-ray source. We argue that these phenomena arise from accretion on to a massive black hole. Possible implications for galactic nuclei in general are briefly discussed.

CENTAURUS A, at a distance of $\sim$5 Mpc, is the closest radio galaxy. Its total radio power is now only $\sim 10^{42}$ erg s^{-1}. The energy content of the very extended radio lobes is, however, $\sim 10^{60}$ erg, and this suggests that Cen A may once have been a powerful radio source of the same type as, for instance, Cygnus A. Recent observations of apparent radio and X-ray variability provide evidence for continuing activity in the nucleus of Cen A. Even though this activity involves a much smaller power output than occurs in QSOs and strong radio sources, it may suggest clues to the nature of active galactic nuclei in general. We discuss here the implications of the new data (particularly the X-ray variability), and argue that they perhaps indicate the presence of a massive ($\gtrsim 10^{7} M_{\odot}$) black hole in Cen A.

Kellermann[1] has detected a central radio component with luminosity $\sim 4 \times 10^{40}$ erg s^{-1} whose spectrum cuts off below $\sim$30 GHz. If the cutoff results from synchrotron self absorption, the size of this component would be only $\sim 10^{16}$ cm. There is some evidence[1,2] for variability on time scales as short as a day.

Cen A is also an X-ray source. Davison *et al.*[3] found that the X-ray intensity had varied over a two-year interval, and therefore proposed that it was associated with a very compact nuclear source. This variability has been confirmed by independent measurements[4-6]; indeed Winkler and White[4] reported X-ray variability of a factor 1.8 ± 0.2 within only 6 d. The spectrum is hard, and there is evidence for variability at photon energies up to $\gtrsim 100$ keV (ref. 7). The X-ray power at <100 keV is $\sim 5 \times 10^{43}$ erg s^{-1}. Hall *et al.*[7] report a γ-ray continuum extending up to ~ 5 MeV, with possible lines at 1.6 MeV and 4.5 MeV; and very high energy ($\gtrsim 300$ GeV) γ rays have been claimed[8].

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Previous discussions of the nature of the X-ray source in Cen A have concentrated on non-thermal models[9-11], and do not specify the source of the necessary relativistic electrons. The idea that massive black holes may lurk in galactic nuclei[12,13] is appealing for several reasons. A black hole seems the likely endpoint for the evolution of a supermassive star or dense star cluster (these being two alternative models for an active galactic nucleus) and accretion on to a black hole can itself account for various phenomena associated with galactic nuclei. If it (or its progenitor) had generated the entire energy content of the extended radio source, the black hole in Cen A would be $\gtrsim 10^7 M_\odot$. In what follows we consider a hole of mass 10^7 M_7 solar masses (and Schwarzschild radius $r_S \simeq 3 \times 10^{12}$ M_7 cm).

Accretion on to massive black holes

If the X rays are thermal bremsstrahlung, then the hard spectrum implies that the temperature is $T \simeq 10^9$ K (in reality, of course, one might expect different parts of the source to span a range of temperature). If the emission comes from gas occupying a sphere of radius r around the black hole, with a volume 'filling factor' f, then the approximate mean particle density n must be

$$n \simeq 10^{14}\left(\frac{r}{r_S}\right)^{-3/2} f^{-1/2} M_7^{3/2}\ \mathrm{cm}^{-3} \tag{1}$$

The ratio of the bremsstrahlung cooling time

$$t_{\mathrm{br}} \simeq 3 \times 10^{11} T^{1/2} n^{-1}\ \mathrm{s}$$

to the free fall time scale

$$t_{\mathrm{ff}} \simeq 10^2 M_7 \left(\frac{r}{r_S}\right)^{3/2}\ \mathrm{s}$$

is then

$$t_{\mathrm{br}}/t_{\mathrm{ff}} \simeq f^{1/2} M_7^{1/2} \left(\frac{T}{10^9\ \mathrm{K}}\right)^{1/2} \tag{2}$$

For the parameters appropriate to Cen A, this ratio never greatly exceeds unity (and it can be made $\ll 1$ by taking a small filling factor); so the efficiencies are adequate provided that the gas can be heated to $T \geqslant 10^9$ K.

If ε denotes the efficiency of the accretion process in converting rest mass into X rays, then the required mass inflow rate is $\sim 10^{-3}\ \varepsilon^{-1}\ M_\odot\ \mathrm{yr}^{-1}$. The corresponding particle density, at distance r from the hole, would be

$$n(r) \simeq 3 \times 10^{11} \varepsilon^{-1} f^{-1} \left(\frac{r}{r_S}\right)^{-3/2} M_7^{-2} \left(\frac{v_r}{v_{\mathrm{ff}}}\right)^{-1}\ \mathrm{cm}^{-3} \tag{3}$$

If transverse motions or pressure gradients slow down the inward velocity, so that the radial velocity v_r becomes $\ll v_{\mathrm{ff}}$, note that n is correspondingly higher for a given mass accretion rate. Equations (1) and (3) cannot be compared directly, since v_r, f and T are probably all functions of radial distance r. They do indicate however that even radially infalling material may produce the observed X rays if $\varepsilon \sim 10^{-3}$, $f \sim 10^{-1}$ and $M_7 \sim 1$.

When the specific angular momentum of the captured material greatly exceeds $a_{\mathrm{crit}} = 2cr_S$, we expect the formation of a significant accretion disk extending well beyond $6r_S$; and it is well known that in this situation a high radiative efficiency $\varepsilon \simeq 0.1$ is almost guaranteed. The 'standard' model[14], however, then predicts disk temperatures of only $T \lesssim 10^5$ K for $M_7 \gtrsim 1$ (no X rays at all). This is because the required luminosity is far below the 'critical' luminosity $\sim 1.3 \times 10^{45} M_7$ erg s^{-1} for such a massive black hole. The inner, radiation pressure supported, region of the disk is therefore thin. The factors f and v_r/v_{ff} in equation (3) are then both very small, and n becomes so high that bremsstrahlung is efficient even at quite low temperatures.

The other well studied type of accretion is that where the inflow is radial (zero angular momentum) and laminar. We see from equation (2) that $t_{\mathrm{br}}/t_{\mathrm{ff}}$ increases with T. The problem here is therefore that if this factor is to be $\lesssim 1$ near the hole, it would have been $\ll 1$ at larger radii. The gas would therefore have remained cool, and the radiative output derived from '$p\mathrm{d}V$ work' would be low[15]. (We avoid introducing the usual concept of 'accretion radius' in this context, because, (1) at large distances the gravitational field of the whole galactic nucleus dominates that of the hole itself, and (2) most of the gas may in fact be produced at a radius where the escape velocity is supersonic.)

Quasi-spherical accretion

Of course there are many ways in which the above models could be modified to account for reasonably efficient production of hard X rays. For instance, realistic disks may involve hot coronae, magnetic flares, and so on. Alternatively, as emphasised by Mészáros[16], continual dissipative heating from turbulence or magnetic field reconnection could raise the efficiency and temperature in the radial inflow picture; but, where cooling is efficient, this model is still constrained by the requirement that

$$kT(r) \lesssim 10^{12} \left(\frac{r}{r_S}\right)^{-1} \frac{t_{\mathrm{br}}}{t_{\mathrm{ff}}}$$

We wish to point out, however, that the X-ray data are most readily explained by an 'intermediate' model where the infalling material has a small but significant specific angular momentum a in the range (say) $1\text{–}30 \times a_{\mathrm{crit}}$. A typical element of gas must then lose its excess angular momentum before it can fall into the hole. (This type of process can in principle yield higher efficiencies than disk accretion. Consider, for instance, two identical (and very small) gas clouds dropping towards a Schwarzschild black hole in orbits having equal opposite angular momentum. If these clouds collided at the point r_{min} when their orbits pass closest to the hole they would come momentarily to rest. If the energy of impact were radiated away, the efficiency would be $1-[1-(r_S/r)]^{1/2}$. For clouds with the critical angular momentum, a_{crit}, the corresponding volume of r_{min} is $2r_S$. This yields an efficiency of 29% (neglecting a correction for the fraction of radiation that would itself be swallowed).)

We can envisage two possibilities: firstly, the accreted material may all have the same sense of rotation. In this case a disk will initially form at a radius $\sim(\langle a \rangle/a_{\mathrm{crit}})^2 r_S$. If the flow continues steadily for a long time, then viscous effects in the disk may cause its rim to grow outwards, but (except in the plane of the disk) inflowing gas would still join the disk after passing through a shock at radius $\sim(\langle a \rangle/a_{\mathrm{crit}})^2 r_S$. Secondly, even if there were no steady systematic rotation, a unit mass of infalling gas might have some angular momentum $\sim a$ arising from small random transverse velocities far from the hole. If $a > a_{\mathrm{crit}}$, this material could not be swallowed until it had lost its angular momentum by colliding with counter-rotating fluid elements near the hole.

In either of the above cases, infalling material will be shocked at a radius $r_{\rm shock} \simeq (\langle a \rangle / a_{\rm crit})^2 r_{\rm s}$, the temperatures attained being $\sim 10^{12}\,(r_{\rm s}/r_{\rm shock})$ K. Thus if $r_{\rm shock} \lesssim 10^3 r_{\rm s}$ the temperatures would be high enough to produce a bremsstrahlung spectrum extending up to photon energies of at least 100 keV. Moreover if $t_{\rm br} \lesssim t_{\rm ff}$ the shocked gas will have time to cool down before being swallowed, thus ensuring reasonable efficiency. The 'impulsive' form of heating from shocks can in this case result in much higher temperatures than from a continual dissipative release of energy.

Most of our discussion is independent of which of these two alternatives applies, and is therefore insensitive to the actual origin of the accreted gas. One interesting possibility is that the gas results from tidal breakup of stars passing close to the black hole[13]. This mechanism can plausibly provide an adequate supply of material; moreover this material would necessarily have angular momentum corresponding to orbits near the 'tidal radius' of the black hole—$\lesssim 10 a_{\rm crit}$ for appropriate parameters[13].

The variability and spectrum of the X rays would, in this scheme, be determined by the details of the inflow, which in turn depends on the origin of the accreted gas. If $M_7 \simeq 1$–10 there is, however, no problem with accounting for variability on any time scale down to hours.

For the spectrum to extend into the MeV band, shocks would need to occur where $r/r_{\rm s} \lesssim 100$. Note that there would then be additional cooling due to electron bremsstrahlung, pair production and 'Comptonisation'. These processes would reduce the cooling time, thus improving the efficiency and strengthening the need for impulsive rather than dissipative heating.

According to Hall *et al.*[7], the spectrum extends up to ~ 5 MeV, and this permits us to set a tentative rough lower limit of $r \gtrsim 10^{15}$ cm to the source dimensions. This limit (which is somewhat geometry dependent) corresponds to the requirement that the 'column density' of hard X-ray photons in the source should not be so high that the γ rays produce e^-–e^+ pairs before escaping[17].

The radio source in the nucleus

The (probably variable) radio component, whose dimensions are also 10^{15}–10^{16} cm, may well be associated with the region that emits the variable X rays. Indeed, the same shock waves which heat the infalling gas may also generate the relativistic electrons. This possibility cannot be explored quantitatively without estimating the magnetic field structure in the gas, or discussing acceleration mechanisms. One can, however, exploit an analogy with supernova remnants such as Cas A, where shock waves involving velocities somewhat lower than we envisage have certainly converted $\gtrsim 1\%$ of the available energy into relativistic electrons. The very high energy γ rays reported by Grindlay *et al.*[8] are presumably somehow associated with the non-thermal radio source.

Radio variability on a time scale of months or years is a well known occurrence in many QSOs, and in the nuclei of some strong radio galaxies. It is commonly interpreted as a succession of 'outbursts', which each produce an expanding cloud of relativistic plasma. VLBI measurements provide evidence for changing structure in these components, and it has been widely argued[18,19] that the outbursts represent successive supernova-style events each releasing $\sim 10^{53}$ erg· the inferred energy per outburst, the event rate (~ 1 yr^{-1}) and the mean luminosity of the galactic nucleus are all compatible with this view. Kellermann[1] emphasises that the nucleus of Cen A resembles a scaled-down version of these other variable sources. The event rate is, however, higher and the mean power output much lower. It seems implausible that supernovae are going off at a rate of 1 d^{-1} in Cen A, which is an order of magnitude less luminous than QSOs. Instead, it seems that the radio outbursts in active nuclei involve flares whose energy is correlated with the mean luminosity (as expected in an accretion picture), rather than supporting a 'building block' model where the energy per outburst is standardised. Moreover, we have shown that the massive black-hole hypothesis accounts naturally for the emission of variable thermal X rays with a hard spectrum. (That 3C279 produced optical flares involving $\gtrsim 10^{54}$ erg (ref. 20) raises problems for the multiple-supernova model in the opposite direction.)

If the nucleus of Cen A involves supernova-type events in a dense star cluster massive and long lived enough to have powered the extended radio source, then the present activity should be spread over a ~ 1 pc region. This corresponds to an angle $\sim 0.04''$—large by the standards of VLBI techniques. Thus if the radio source, when it has been observed for a longer interval, fails to display any 'jitter', this would support the kind of model proposed here involving a massive black hole. It would not, of course, preclude the possibility that a dense star cluster is indirectly involved, as envisaged by Hills[13].

Discussion

Accretion of material with low, but not negligible, specific angular momentum by a massive black hole can be an efficient means of producing hard X rays. Mészáros[16] has emphasised that dissipative heating may greatly enhance the efficiency when $a < a_{\rm crit}$, and we find that shocks involving velocity discontinuities of $\gtrsim 0.1c$ can occur in the intermediate case of $a_{\rm crit} < a < 30 a_{\rm crit}$. Radiation from behind such shocks can produce a hard spectrum extending up to $\gtrsim 1$ MeV. This mechanism can account for the observed X-ray emission from the nucleus of Cen A. The variability in the X-ray source may arise from changes in the mass flow rate, or amount and distribution of angular momentum in the infalling gas. (An analogous model may be relevant to Cygnus X-1 (refs 21–23).) 'Standard' accretion models[14,15] fail to account for hard X-ray emission from massive black holes.

A similar model may also apply to the nucleus of 3C390.3, which is possibly a variable X-ray source[24], and such objects as NGC4151 and 3C273. The strong X-ray absorption observed[25] in these last two suggest again that the nucleus is responsible. An alternative scheme involving multiple stellar mass 'events' in a dense star cluster might be tested by searching for jitter with VLBI techniques. It is also possible in this case that the X-ray absorption might change if a substantial fraction of the absorbing material lies in the star cluster. If the analogy between Cen A and more powerful galactic nuclei is genuine, the implications for theories of extragalactic violent activity in general would be basic and fundamental.

D. M. acknowledges an Academia dei Lincei–Royal Society Fellowship.

Received February 12, accepted March 11, 1976

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Source of terrestrial non-thermal radiation

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Observations from satellites have revealed the existence of continuum radiation from just beyond the plasmapause. I propose here that this is generated by Cerenkov radiation coupling to the O mode of radiation, which can propagate in low density plasmas. The observations are interpreted on the basis of this theory.

RECENT observations by spacecraft of radio noise at distances beyond about six Earth radii (R_E) from the Earth indicate the presence of non-thermal continuum radiation (ref. 1, and D. A. Gurnett, unpublished) in the frequency range 500 Hz–100 kHz. Direction finding measurements on the Imp 6 spacecraft show that the radiation is generated in the outer radiation zone immediately beyond the plasmapause boundary[1]. The source region extends from 0400 to 1400 (LT) and covers a broad latitude range, which includes the geomagnetic equator (D. A. Gurnett, unpublished); a separate source exists on the morning side of the magnetosheath.

The radiation has a sharp lower frequency cut-off near to the local electron plasma frequency, f_p, which is proportional to the square root of the electron density in the vicinity of the spacecraft; f_p ranges from 500 Hz in the distant magnetotail to >100 kHz near the plasmapause. No distinct high frequency cut-off is observable and the intensity decreases as the frequency increases; > 100 kHz the radiation disappears below the cosmic noise level. The continuum seems to consist of two components, one which fills the whole magnetosphere and magnetotail and which is permanently trapped between the plasmasphere and magnetosheath, and another component which can penetrate the magnetosheath to propagate freely into the solar wind[2,3].

A spectrogram showing the low frequency cut-off of the radiation when Imp 6 was situated at ~8R_E and 0545 (LT) is seen in Fig. 1, where the picture intensity is proportional to the amplitude of the noise. The signals from the electric and magnetic antennae are shown alternately; the magnetic signal appears weaker because the experiment sensitivity to this component is less.

The radiation is seen to have two distinct cut-offs, one at frequency $f_1 \simeq 5.3$ kHz and the other at $f_2 \simeq 6.35$ kHz. In the range between f_1 and f_2 the noise varies periodically as the spacecraft rotates, the nulls in the observed electric component occurring when the electric field vector of the radiation is perpendicular to the dipole antenna used for reception. It is stated that this electric field is polarised predominantly parallel to the geomagnetic field[1]. At frequencies $>f_2$, bands of noise are apparent at ~7.7 kHz, 8.6 kHz and 9.4 kHz. These bands are not always observed but seem to appear irregularly when the spacecraft is close to the plasmapause. Since they are observed on both electric and magnetic channels they are apparently electromagnetic in character.

Mechanisms which have been proposed as a source of the continuum radiation are gyrosynchroton radiation[3] and electrostatic instabilities at half harmonics of the electron gyrofrequency[1], that is at $(n+\frac{1}{2})f_g$ where n is an integer and f_g is the gyrofrequency (which is proportional to the magnetic field strength). There is recent evidence (D A. Gurnett, unpublished) that the radiation is dependent on the flux of particles of energy <30 keV and this tends to eliminate the gyrosynchroton mechanism which requires particles of much higher energy, ~1 MeV. The second mechanism proposed—electrostatic instabilities—requires that the energy be transferred from electrostatic waves to electromagnetic waves and it is suggested that this can occur if the medium is inhomogeneous.

I here propose that the observed radiation is produced originally by the Cerenkov mechanism as electromagnetic waves in a mode that cannot normally propagate over large distances in the magnetosphere. Close to the source region, however, these waves may become coupled to electromagnetic waves in another mode which can propagate over large distances.

Theory

Cerenkov radiation is produced when particles are moving through a medium with velocities greater than the phase velocity of the radiation in the same direction. Since particle velocities cannot exceed the velocity, c, of electromagnetic radiation in free space, the Cerenkov criterion requires that the wave refractive index be greater than unity; the lower the speed of the particle, the larger the refractive index required.

Figure 2 illustrates the variation of the square of the refractive index (n^2) with X, when $Y < 1$ and collisions and temperature effects are neglected[4]; the parameters X and Y are as normally defined in magnetoionic theory[5]

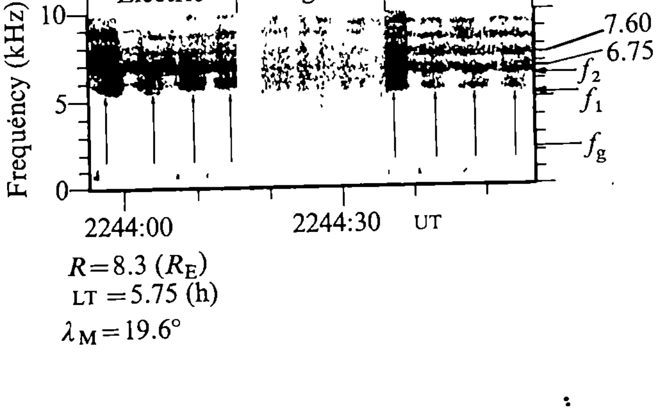
$$X = f_p{}^2/f^2; \qquad Y = f_g/f,$$

where f is the wave frequency and f_p, f_g are the plasma frequency and gyrofrequency respectively. The condition $Y < 1$ requires that $f > f_g$; this condition and the assumption that collisions can be neglected are satisfied for wave frequencies >5 kHz at radial distances of $\geq 6R_E$.

It is apparent from Fig. 2 that three modes of propagation are possible for the range of X shown. The mode whose refractive index goes to zero at (the mode with a cut-off at) $X = 1 - Y$ is the first extraordinary mode—the X mode. The mode with a cut-off at $X = 1$ is the ordinary (O) mode, and the mode having a cut-off at $X = 1 + Y$ is the second extraordinary mode, usually termed the Z mode. The shaded regions indicate where the curves of n^2 must lie as the angle θ between the wave normal direction and the magnetic field direction is varied; the full lines labelled a, b and c show the curves corresponding to some wave-normal angle θ intermediate to 0 and $\pi/2$.

It is important to note that as $\theta \rightarrow 0$, when the wave normal and magnetic field directions tend to be parallel (or antiparallel),

Fig. 1 v.l f Spectrogram obtained on Imp 6 (from ref. 1). Arrows indicate electric antenna parallel to geomagnetic field.



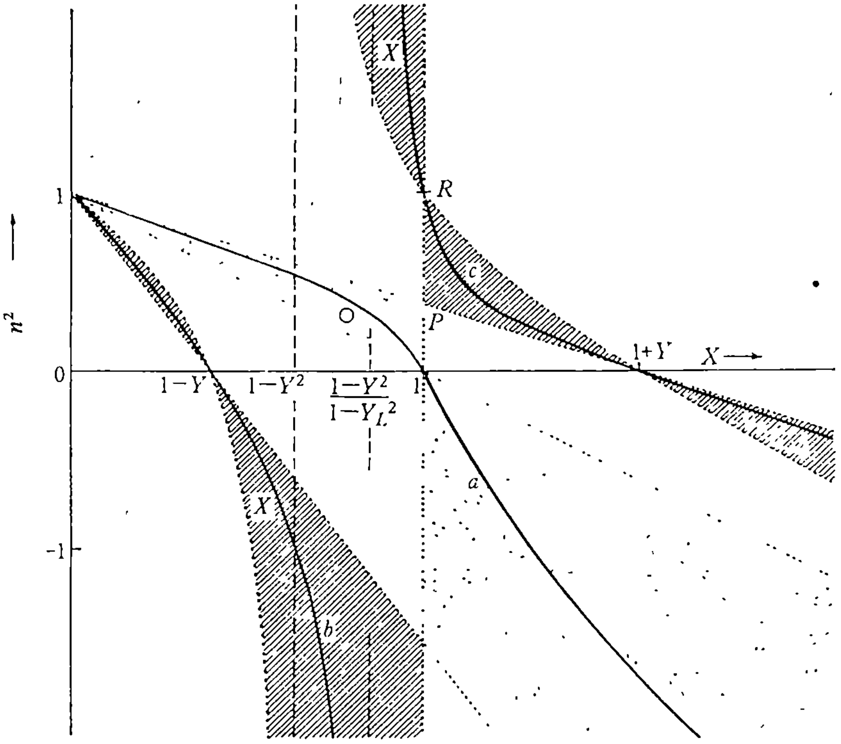


Fig. 2 Variation of (refractive index)^2 with X when $Y<1$ (after Budden[4]). Electron collisions are neglected.

the curves a and c approach the point P at $X = 1$. The refractive index for the Z mode increases rapidly $< X = 1$ and $\rightarrow\infty$ at $X = (1-Y^2)/(1-Y_L^2)$ where $Y_L = Y\cos\theta$. As $\theta \rightarrow 0$, $Y_L \rightarrow Y$ and the infinity in $n \rightarrow X = 1$; as $\theta \rightarrow \pi/2$, $Y_L \rightarrow 0$ and the infinity $\rightarrow X = 1-Y^2$. This infinity is termed the upper hybrid resonance (UHR).

The Cerenkov criterion can be satisfied only in the region $1-Y^2<X<1$ since it is only there that $n>1$. Observations on rockets and satellites in the ionosphere and magnetosphere[6-16] show that under certain conditions, enhanced radiation, which has been called UHR noise, does exist in this frequency range extending from the plasma frequency $f = f_p$ ($X = 1$), to the UHR frequency $f = (f_p^2+f_g^2)^{1/2}$. Calculations show that Cerenkov radiation from typical magnetospheric energy spectra is adequate to provide the power observed in UHR noise[17]. With reference to Fig. 2, it is clear that UHR noise cannot escape directly to regions of lower plasma density (smaller X) since the radiation is absorbed at the UHR. If, however, the Z waves propagate first into regions of increasing plasma density and reach the level $X = 1$ where the wave frequency becomes equal to the plasma frequency, then it is possible in certain conditions for the energy to be transferred or 'coupled' from the Z mode to the O mode. The O-mode energy is then free to propagate to lower values of X as is apparent from curve a in Fig. 2.

The theory of coupling[4] yields a coupling parameter ψ given by the expression

$$\psi = -\frac{iY_T^2Y_L}{4}\frac{X^1+iZ^1}{(1-X-iZ)^2Y_L^2+(Y_T^4/4)} \qquad (1)$$

where $Y_T = Y\sin\theta$ and Z is the ratio of collision frequency to wave frequency and must not be confused with the Z mode. The parameters X^1 and Z^1 are the spatial gradients of X and Z respectively It is seen from equation (1) that ψ can become large if simultaneously $X\rightarrow 1$ and $Z\rightarrow(1/2)|Y_T^2/Y_L|$. These are the conditions for 'critical coupling'. In the magnetosphere beyond a few thousand kilometres altitude, the value of Z computed on the basis of Coulomb collisions is small and thus critical coupling is achieved if $Y_L \gg Y_T$ at $X = 1$. Therefore, one can expect energy transfer from the X mode to the O mode near $X = 1$ if the wave normal direction is close to the magnetic field line direction; in this condition curves a and c in Fig. 2 approach one another at point P.

Further theory

To understand in more detail the emission process and the consequent wave propagation and coupling, it is necessary to consider the refractive index surfaces (n surfaces). An n surface is a surface on which lies the tip of a vector whose direction is that of the wave normal and whose magnitude is proportional to the phase refractive index for that direction. The n surfaces for the Z mode are illustrated in Fig. 3a. For a given value of Y, a family of n surfaces may be drawn corresponding to different values of X. The surfaces are in fact surfaces of revolution about the geomagnetic field $\mathbf{B}_0$

To determine graphically the wave normal angle at which Cerenkov radiation is produced by a particle with velocity $c\beta$ along the magnetic field, $\beta<1$, a line may be drawn normal to $\mathbf{B}_0$ at a distance $1/\beta$ from the origin. Where this line intersects an n surface—for example at M in Fig. 3a—the Cerenkov condition is satisfied; the radiation produced at the level corresponding to $X = 0.9$ will then have its wave normal along **OM**. Note that for $X<1$ the shape of the n surfaces is such that particles of all energies (all values of β) will produce waves all of which have nearly the same wave normal direction. In practice, temperature effects limit n to finite values, so that the lower energy particles do not contribute to the power radiated. After the waves are produced, propagation into regions of lower or higher X may be followed by using Poeverlein's construction. This is based on Snell's law, which requires that the tangential components n be equal above and below an isoionic plane (plane of constant X) through which the wave travels[7]. In Fig. 3a consider the isoionic plane AA' which has been selected so that its normal passes through points M and P; the significance of this will appear later. The original wave normal direction is along **OM** but the group velocity of the wave is in the direction perpendicular to the n surface at M (see Budden[4]) as indicated by the small arrow; this is the ray direction. If the energy propagates into regions of increasing electron density (increasing X) the locus of the tip of the wave normal moves from n surface to n surface along the line MN. The ray path resulting from the condition that the ray direction should

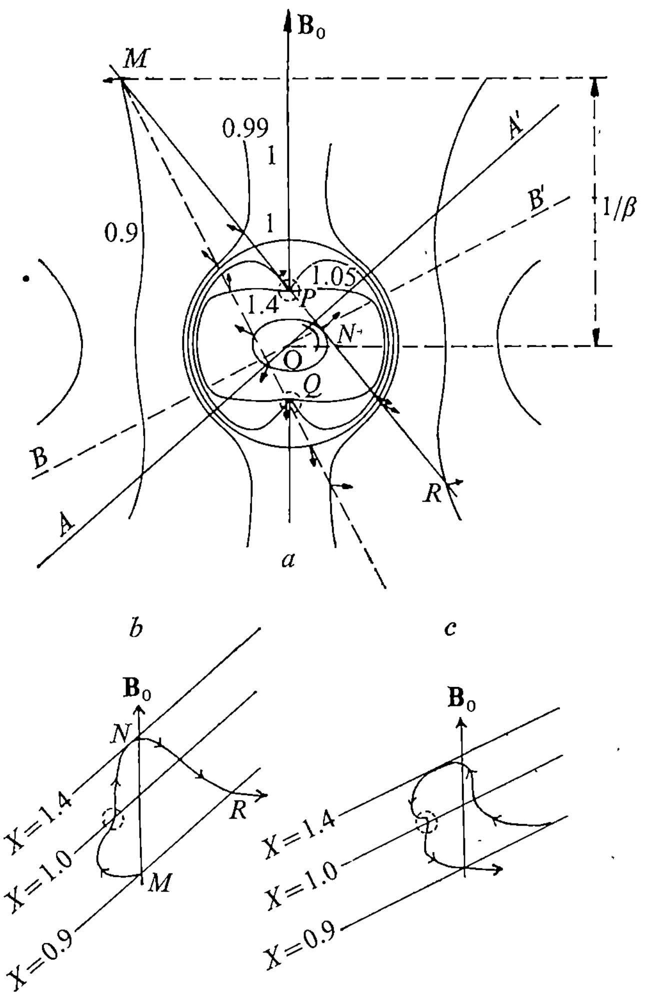


Fig. 3 *a*, Refractive index surfaces for the *X*-wave when $Y<1$. Figures on the curves are values of *X*. *b*, Ray path with isoionic contours *AA'*. *c*, Ray path with isoionic contours *BB'* The dotted circles indicate the regions of coupling.

always be normal to the *n* surfaces at the tip of the wave normal is shown in Fig. 3*b*. At point *N* the wave is reflected, its wave normal following the line **NR** in Fig. 3*a* and its ray following *NR* in Fig. 3*b*.

It is evident from Fig. 3*a* that when the waves pass through the region corresponding to $X = 1$, the wave normal becomes aligned with $\mathbf{B}_0$ and the two conditions for critical coupling are satisfied. Energy can be transferred at this point from the *Z* wave to the *O* wave whose *n* surface at this level also passes through point *P* as can be deduced from Fig. 2 (see also Budden[4]). The *O* wave so produced cannot propagate into regions of higher *X* (see Fig. 1) but is immediately reflected to propagate unhindered to regions of lower plasma density. A similar process occurs if the isoionic planes are aligned parallel to *BB'*, except that in this case the coupling occurs near $X = 1$ after the *Z* wave has been reflected; the corresponding ray path is shown in Fig. 3*c*.

The significance of the alignment of *AA'* and *BB'* is now clear —these are the orientations of the isoionic planes which render the coupling most efficient. It is seen from equation 1 that the coupling parameter ψ is also dependent on X^1 and therefore if X^1 is large one may expect some coupling to occur, even when the isoionic planes are not strictly aligned to *AA'* or *BB'*. Calculating the actual amount of coupling for different wave normal angles is the subject of further study.

Interpretation of observations

On the basis of the mechanism proposed, the frequency cut-offs appearing in the Imp 6 spectrogram (Fig. 1) can be identified. The lower cut-off at f_1 corresponds to the *Z*-mode cut-off ($X = 1+Y$) at the spacecraft; frequencies $<f_1$ will not reach the spacecraft, since they encounter cut-offs in the lower density regions above the spacecraft altitude. In fact, if f_1 is to correspond exactly to the condition $X = 1+Y$ at the spacecraft, some waves of this frequency must be incident normally on the isoionic plane, since waves incident obliquely will be reflected before reaching $X = 1+Y$. The Cerenkov radiation is expected to be produced with a fairly large range of wave normal angles since usually a wide range of particle energies is involved; the effect on the wave normal angle of varying β may be deduced from Fig. 3*a*. It is expected, therefore, that there will be a sufficiently broad distribution of wave normal angles to ensure that some of the waves come very close to the level $X = 1+Y$ before being reflected.

The other frequency cut-off at f_2 may be identified with the level $X = 1$ where the *O* wave is produced. At this level the *O* waves will have their normals mainly aligned to the geomagnetic field, this being a condition for their production. Thus one does not have a broad distribution of wave normal angles as for the *Z* mode and, theoretically, reflection of the *O* wave will occur before the level $X = 1$ is reached for the case shown in Fig. 3*b*. It is probable, however, that the region of coupling is so close to $X = 1$ that the cut-off at f_2 can be attributed to the *O*-wave cut-off at $X = 1$. This is also true if the coupling occurs after *X*-wave reflection (Fig. 3*c*).

From the identification of the cut-off frequency $f_2 = f_p = 6.35$ kHz, the plasma density at the spacecraft is calculated to be 0.5 electrons cm^{-3}, and if one uses the relation $X = f_p^2/f_1^2 = 1+Y = 1+f_g/f_1$, the electron gyrofrequency f_g at the spacecraft is found to be 2.3 kHz. The value of f_g at the spacecraft calculated from a harmonic expansion of the geomagnetic field is 2.2 kHz. A previous identification[1] assumed f_1 to be the plasma frequency and f_2 to correspond to the *X*-wave cut-off at $X = 1-Y$ (see Fig. 2). This resulted in an estimate of electron density of 0.35 electrons cm^{-3} and a gyrofrequency of 1.9 kHz. The maximum possible error from such a misidentification would amount to a factor of 2 in plasma density.

In the frequency range between f_1 and f_2, the electric field of the radiation should be predominantly perpendicular to the static magnetic field with the longitudinal component increasing rather rapidly near the plasma frequency[18,19]. With a single dipole antenna it is difficult to determine unambiguously the orientation of the electric field vector when the wave is linearly polarised, and a more detailed investigation of the spin-modulated noise between f_1 and f_2 using three mutually orthogonal antennae is required.

From the new identification of cut-offs presented here, the UHR frequency at the spacecraft is calculated to be $f_{\mathrm{UHR}} = 6.75$ kHz and the cut-off of the *X* wave should be at $f_X = 7.6$ kHz. These frequencies are indicated in Fig. 1, and they are seen to correspond to abrupt changes in noise intensity. The larger intensity <6.75 kHz is understandable since it is in this range $f_2<f<f_{\mathrm{UHR}}$ that the noise is believed to be produced by the Cerenkov mechanism. The fact that the intensity change occurs very close to the UHR, indicates that these waves have large wave normal angles, otherwise the intensity would have changed at a lower frequency corresponding to $X = (1-Y^2)/(1-Y_L^2)$ as may be deduced from Fig. 2.

It is tempting to identify the increase in intensity >7.6 kHz as being due to the presence of the *X* wave. There is no known mechanism for producing this mode locally but it is possible that it could have been formed by depolarisation of the *O* wave by reflections at the magnetosheath and at magnetospheric irregularities in plasma density. The resulting *X* waves could then propagate inwards and be reflected at the level $X = 1-Y$, thus producing an abrupt change in noise intensity at 7.6 kHz. The noise bands at higher frequencies would, however, remain unexplained.

An explanation of the noise bands usually observed close to the plasmapause as being harmonics of the local gyrofrequency has been ruled out, since the difference in frequency between bands in Fig. 1 is ~ 1 kHz whereas the gyrofrequency is > 2 kHz. It also seems that the noise bands are not accurately harmonically related. One possible explanation for these bands is that they are produced as a consequence of ionisation ducts below the spacecraft altitude. As is seen from equation 1, plasma density gradients within ducts would render coupling more efficient, X^1 being larger, and one could expect bands of more intense radiation at frequencies corresponding to the duct plasma densities. On this assumption and by using an average plasma density profile[1] for the magnetosphere, one can estimate the distance between ducts below the spacecraft to be $\sim 0.3 R_E$. It is significant that ducts are known to exist predominantly within the plasmasphere and the noise bands are usually seen close to the plasmapause.

Conclusions

It is concluded that mode coupling of Cerenkov radiation may be the source of terrestrial non-thermal radiation. The mechanism requires that the plasma be traversed by energetic particles and this is known to be the case in the magnetosphere. The angle between the geomagnetic field and the isoionic planes is an important parameter and this may explain the local-time asymmetry in the source location, since it is well known that the plasmasphere has a distinct outward bulge at local evening. In addition, the dawn–dusk magnetospheric electric field increases the local-morning electron energies (D. A. Gurnett, unpublished) by ~10 keV and this has obvious implications on the Cerenkov mechanism proposed.

The radiation which escapes to regions of lower plasma density will be in the ordinary mode which is left-hand polarised; polarisation measurements of the noise are not yet available. The radiation whose frequency is greater than the magnetosheath plasma frequency will escape into the solar wind; the radiation whose frequency is less than the sheath plasma frequency will be reflected and will be trapped between the higher plasma densities of the sheath and plasmasphere, ultimately escaping down into the magnetotail.

Conditions suitable for the mechanism proposed also exist in the morning magnetosheath, which is a favoured region for streaming electrons from the solar wind[20,21].

Received June 9, 1975, accepted February 2, 1976

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letters to nature

Type I supernovae and galactic production of iron

CURRENT models of galactic chemical evolution (see ref. 1) assume that heavy elements are produced by massive stars, that is, in type II supernova events. We show here that Fe may have a different source from other heavy elements—in type I supernovae which result from the deaths of moderately long lived stars.

Type I and type II supernovae both have similar radii and temperatures near maximum light[2], yet the spectra of type I supernovae show considerably more structure. Many of the lines have been attributed to Fe[3]. At a time long after maximum light, Kirshner and Oke[4] were able to study the emission from SN 1972e in NGC 5253 in a quantitative way. If their identification of Fe II lines is correct, $\sim 0.03 M_\odot$ of Fe is required. This is a lower limit to the Fe abundance and provides strong observational evidence for Fe production in type I supernovae.

Observations of type II supernovae at around maximum light do not show any overabundances of heavy elements. This is easily understood since the envelope of the presupernova star is being observed. The only observable material which has been recently processed by a massive star may be the fast moving knots in Cas A (refs 5, 6). Only lines of Ar, O, and S have been detected; no Fe lines have been observed although Fe II lines might be expected. No attempt has yet been made to put a limit on the Fe abundance. Thus, while there is no observational evidence for Fe production in type II supernovae, there is no evidence to the contrary. Stellar evolution calculations do not clarify the problem since it is unknown how much Fe is contained in the possible compact remnant of a supernova.

The last piece of evidence on Fe production comes from the theory of supernova light curves. First consider the supernova energy to be deposited instantaneously. If the observed supernova luminosity near maximum light is to be reproduced, the radius of the presupernova star must be $\geqslant 10^{14}$ cm (refs 7, 8). This condition is satisfied for massive stars, but may not be the case for the small mass progenitors of type I supernovae. If the initial radius is small, some supernova energy must be stored over tens of days. Colgate and McKee[8] have suggested that the energy is stored in the radioactive products of explosive nucleosynthesis. The model which reproduced the observed magnitude of type I supernovae produced $0.25 M_\odot$ of Fe. If any more Fe is produced explosively, the models predict excessive luminosity. It should be noted that the observed luminosity being reproduced in these models is based on a Hubble constant, H, of 100. A lower value of H would require greater Fe production. Thus, from observations and models of type I supernovae, their Fe yield is in the range of 0.03–$0.25 M_\odot$.

This yield can be compared with that required to give the present solar abundance of Fe. Using solar neighbourhood values for the birthrates of moderate-mass stars and galactic mass density, Ostriker *et al.*[9] derive limits on the Fe production per massive star. It is certainly $< 0.23 M_\odot$ of Fe per star and is very likely to be $< 0.023 M_\odot$ of Fe. The birthrate would be equivalent to the type II supernovae rate. Tammann[10] finds that types I and II supernovae have similar total rates and radial distributions in galaxies like our own. Thus the yield found by Ostriker *et al.* also applies to type I yields.

These considerations indicate that even the observed amount of Fe in type I supernovae may be sufficient to give the Fe abundance in the solar neighbourhood. If the low Fe production per supernova is correct, the radioactivity model for type I supernovae is incorrect, and models with either extended initial envelopes or another form of late energy input must be considered.

Fe production in type I supernovae has implications for the chemical history of the Galaxy. It is generally accepted that type I supernovae result from less massive stars than those giving type II supernovae (for a recent review, see Tinsley[11]). The lifetimes of the pre-type I supernovae stars may be an order of magnitude longer than that of the pre-type II supernovae stars. Assume that the stellar mass function is independent of time. During the initial burst of star formation, there is a lag between the production of Fe in type I supernovae and the production of the other heavy elements in type II supernovae. During this period, stars can be formed which are underabundant in Fe relative to the other heavy elements. There is some evidence that old stars are overabundant in O relative to Fe (ref. 12), but the observed abundances may not reflect the initial composition of these stars. Peimbert[13] notes that the O overabundance may well be primaeval. He also reviews evidence that at least two parameters are required to describe the horizontal branch characteristics of globular clusters and suggests that the two parameters are the Fe abundance and the CNO abundance.

If considerable heavy element processing occurs in the disks of spiral galaxies, the type I supernova origin of Fe is of some importance. Observations of supernovae show that type II supernovae are concentrated in spiral arms while type Is are scattered throughout the disk[14]. A given volume of gas thus receives processed material from type II supernovae in bursts and material from type Is more uniformly. The result is that the ratio of Fe to other heavy elements may be expected to show fluctuations.

Heavy elements are overabundant in cosmic rays in a way that is consistent with the supernova origin of cosmic rays. Ramaty *et al.*[15] found that the energy spectrum of Fe cosmic rays is flatter than that of CNO nuclei. They interpret the result as indicating that Fe nuclei are accelerated near to a pulsar while the other heavy elements are accelerated in the surrounding nebula. The present work suggests a different interpretation: Fe nuclei are accelerated in type I supernovae and CNO nuclei are accelerated in type II supernovae. If a Fermi-type acceleration mechanism is operating, the flatter Fe energy spectrum may arise from the higher material velocities in type I supernovae.

In the future, observational checks on the source of galactic Fe will be possible. Clayton[16] has reviewed arguments that Fe is produced explosively and claims that γ-ray line observations of supernovae will enable abundances of nucleosynthesis products to be determined. Also, young supernova remnants such as the remnant of Tycho's supernova of 1572 should be examined for the presence of Fe emission The supernova ejecta may have been heated to high temperature in a reverse shock wave or a conduction front, so that X-ray observations with high spectral resolution may be the most profitable. Third, observations of extragalactic supernovae, particularly at late times, will be useful. These observations should yield information on whether type I supernovae are important contributors of galactic iron.

The Kitt Peak National Observatory is operated by the Association of Universities for Research in Astronomy Inc., under contract with the NSF.

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Received December 24, 1975, accepted March 16, 1976.

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X-ray emission from γ Cas

THE Copernicus orbiting observatory contains two astronomical experiments, the Princeton ultraviolet spectrometer and the MSSL X-ray package. They are coaligned, and during ultraviolet observations the 3–8 keV collimated proportional counter routinely collects background data[1] When the observatory was pointed at the ultraviolet target γ Cas on December 21, 1975 and again on January 20, 1976, a clear excess of counts was seen in the 3–8 keV channel compared to preceding and following targets. This count rate corresponds to a flux at the Earth of $\sim 1.2\times10^{-10}$ erg cm^{-2} s^{-1}, and lies 5σ above the mean background level determined from several weeks of observations (Table 1)

We have also examined earlier Copernicus X-ray data taken on γ Cas The star was observed on November 4, 1973 and on November 13, 1974. No flux was detected during the 1973 observation, but the sensitivity attainable was low because of the sparsity of background data near that time A signal was detected in 1974, 4σ above the mean background.

The FWHM field of view of the Copernicus X-ray detector is $\sim 3°$, and we cannot localise the emission to greater accuracy than this. Jernigan[2] has, however, reported that the SAS-3 satellite, in a 2-d observation beginning December 30, 1975, observed X-ray emission of comparable intensity to that reported here from a region of sky within 1′ of γ Cas. This suggests a high probability that the X-ray emission originates from the vicinity of this star, although we note that γ Cas does have a companion ADS782B ($m_v \approx 10$), the two stars being separated by ~2″ (ref. 4). The X-ray source was not detected before November 1974; in particular, it was not recorded in the Uhuru sky survey[3,4], which suggests that it is variable. There is no evidence within the Copernicus data for flux variations $>1\sigma$, but the strength reported by the SAS-3 group[2] ($3\ 5\times10^{-10}$ erg cm^{-2} s^{-1} from 2–11 keV) is a factor of ~ 1.5 above the highest value seen by Copernicus, allowing for the difference between the energy range of the two detectors.

γ Cas is an extreme Be star whose magnitude is variable between 1.6 and 3.0 (ref. 4). Its absolute visual magnitude is probably ~ -4 (ref. 4), so that the intrinsic luminosity of the X-ray source is $\sim 10^{32}$ erg s^{-1}. Maraschi, Treves and van den Heuvel[5] have noticed the several coincidences

Table 1 X-ray data from γ Cas

Observations Mid-time	Duration (h)	c.p.m. (3–8 keV)	erg cm^{-2} s^{-1} ($\times 10^{-10}$)
1973 November 4.4	16	<3.0	$<1\ 2$
1974 November 13 7	37	2.0 ± 0.5	0 8
1975 December 21 6	12	$3.3\pm0\ 6$	1 3
1976 January 20 4	22	$2.8\pm0\ 5$	1.1

The uncertainties in counting rate are dominated by systematic background effects which are measured empirically. The energy flux is derived assuming a Crab-like spectrum, but the conversion factor from c p m to erg cm^{-2} s^{-1} is relatively insensitive to spectral slope and form.

between Be stars and X-ray sources. In particular, γ Cas is very similar in its optical characteristics to X Per (ref. 4), which has been associated with the regularly varying X-ray source 3U0352+30 (refs 6–8). The ratio of X-ray to optical luminosity of the two stars now differs by just over an order of magnitude[8]. No evidence is found for regular modulation in the present data between 2 and 40 min. The source is, however, comparatively weak, and any modulation of peak to mean amplitude <60% of the mean flux would have gone undetected. More sensitive observations will be necessary to explore further the possible parallels between γ Cas and X Per Meanwhile, γ Cas provides yet another example of the growing number of intrinsically relatively weak X-ray sources being discovered which are associated with nearby stellar objects (see ref. 9).

We are grateful to P. A. Charles for discussions. K.O.M. and N.E.W. acknowledge support from the SRC.

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Received February 12, accepted March 18, 1976.

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Radiometric diameter and albedo of the remarkable asteroid 1976AA

THE minor planet 1976AA (Fast-Moving Object Helin), discovered on January 7, 1976, is the first asteroid found with an orbital period of < 1 yr (orbital elements $a=0.97$, $e=0.18$; $i=19°$; ref. 1). Although it seems to be a likely speculation that 1976AA is a former Apollo object that originally had its aphelion in the main asteroid belt, it is possible that this object is a member of a group of minor planets that is distinct in physical as well as orbital properties. Observations that reveal the physical nature (colour, albedo, texture, mineralogy) of its surface are needed to compare it with better studied objects in more orthodox orbits. This letter reports a determination by infrared radiometry of the diameter and albedo of 1977AA.

The radiometric technique of measuring diameters and albedos was first applied to asteroids in 1970 (ref. 2) and has recently been discussed in some detail[3,4]. Physically, it is based on the dependence on albedo of the balance between reflected and thermally emitted radiation for an airless body in thermal equilibrium with the sunlight striking it The application reported here follows the formulation and calibration by Jones and Morrison[4], which has previously been used to determine the diameters of > 60 main-belt asteroids[5,6] and the Earth-approaching object 433 Eros[7].

The observations were made on January 26, 1976 in a broad spectral band centred at ~ 10 μm with the infrared photometer[8] of the 1.5-m telescope of the Lunar and Planetary Laboratory Catalina Observatory. The primary stellar standards were α Tau and β Gem, for which we adopted magnitudes of -3.1 and -1.3, consistent with the standard calibration assumed for the radiometric diameter technique[5]. Two groups of integrations, centred at UT dates 26.22 and 26.30, yielded a 10-μm magnitude of $+5.0\pm0.3$. At the time of observation, 1976AA was 0.150 AU from Earth and 1.075 AU from the Sun, and its phase angle was 50°. Although it is not clear exactly how the radiometric brightness should be reduced to full phase, both model calculations and observations of 433 Eros[7] suggest a correction of between -0.01 and -0.02 mag deg^{-1}, yielding an equivalent 10-μm magnitude at zero phase of $+4.3\pm0.5$. The value of $V(1,0)$, the zero-phase, unit-distance visual magnitude, is $+17.6$ from other observations made here (J.C.G., unpublished).

A straightforward solution for diameter and visual geometric albedo based on these observations yields $D=900\pm200$ m and $p_V=0.20\pm0.07$. This albedo is in satisfactory agreement with a preliminary polarimetrically derived value (J. C. Gradie, unpublished), and the diameter is the smallest ever measured for an asteroid.

On the basis of its albedo, 1976AA can probably be classed with the stony or silicaceous (S-class) group of asteroids[9]. These asteroids seem to have related surface mineralogies and to be the predominant type near the inner edge of the belt, although the much darker carbonaceous objects constitute the great majority of all asteroids. The previously studied Mars- and Earth-orbit-crossing objects—433 Eros, 887 Alinda, 1566 Icarus, and 1685 Toro—also belong to the broad S compositional class. Thus, on the basis of this preliminary physical datum, 1976AA appears similar physically to other small asteroids that approach the Earth.

This research was supported in part by grants from NASA. We thank E Helin for informing us of the existence of 1976AA, and J. G. Williams for providing the ephemeris without which these observations could not have been made.

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Received February 10, accepted March 18, 1976.

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On simultaneous tilt and creep observations on the San Andreas Fault

THE installation of an array of tiltmeters along the San Andreas Fault[1] has provided an excellent opportunity to study the amplitude and spatial scale of the tilt fields associated with fault creep. We report here preliminary results from, and some implications of, a search for interrelated surface tilts and creep event observations at four pairs of tiltmeters and creepmeters along an active 20-km stretch of the San Andreas Fault. We have observed clear creep-related tilts above the instrument resolution (10^{-8} rad) only on a tiltmeter less than 0.5 km from the fault. The tilt events always preceded surface creep observations by 2–12 min, and were not purely transient in character

Although episodic fault creep has been observed at many locations of the San Andreas, Hayward, Calaveras, and other active faults in the San Andreas Fault System[2–8],

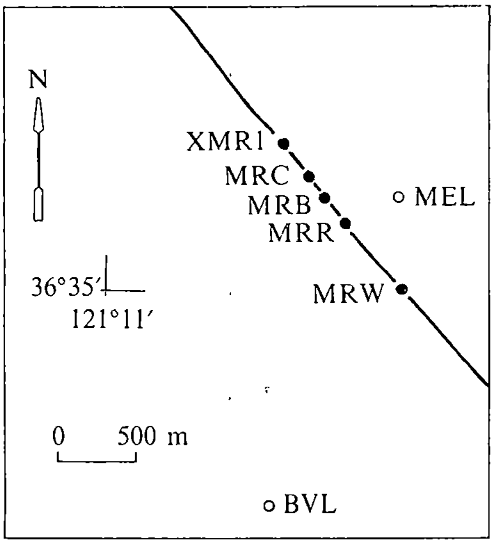

Fig. 1 Location map in the Bear Valley region of central California of tiltmeters (MEL and BVL) and creepmeters (MRW, MRR, MRB, MRC and XMR1) along the San Andreas Fault.

measurements of transient tilts and strains associated with creep events have not yet been made in sufficient detail to allow estimates of critical parameters such as depth, dimension, amount of slip, and the effects of material property variations. These parameters are critical to questions concerning, first, the degree to which the dynamic aseismic slip behaviour of the fault is reflected in fault creep measurements; second the relationship between observed creep and seismicity; and third, the source of the nonlinear form of the creep signals. Typical creep events have amplitudes of a few millimetres and durations of a few minutes to a few days[8,9]. Fault displacements inferred from cumulative creep[10] are in approximate agreement with those measured using geodetic techniques[11], and approximately simultaneous observations of creep events at closely spaced creepmeter sites have been interpreted as slip propagation along the fault at rates of up to 10 km d^{-1} (refs 4 and 6).

With one exception, the tiltmeters considered here form part of an array of shallow borehole instruments approximately 6 km apart, and 1–2 km from the fault (Fig. 1), intended primarily for the detection of earthquake related deformation[1] The exception is the tiltmeter at Melendy Ranch (MEL) which was installed 370 m from the fault near several Melendy Ranch creepmeters This instrument grouping, in addition to tiltmeter MEL on the north-east side of the fault, consists of creepmeters XMR1, MRC, MRB, MRR, and MRW along the fault from north-west to south-east, and tiltmeter BVL on the south-west side of the fault, as shown in Fig 1. At these creepmeters clear examples of episodic creep on the San Andreas Fault are observed every few months[8,9].

Since January, 1974, three of the largest creep events, each recorded on at least two creepmeters, have occurred while both tiltmeters were operating. Figure 2 shows about 6 h of parallel record from the two tiltmeters and the particular creepmeters in operation at the time of these creep events The uncertainty in timing for all sets of records is less than 2 min.

For the first creep sequence of July 11, 1974 (Fig. 2*a*) creep was recorded only on meters MRC and XMR1 to the north-west of MEL The times of onset were 1745 and 1808 GMT, respectively. For more than an hour before 1743 a ramp-like change is evident in the north–south tilt component at MEL (Fig 2*a*). Although interesting, this is the only example of a tilt ramp of this sort, and its meaning is not clear. From 1743 to 1800 the tilt at MEL increased by 0 5 μrad in a direction S41°W. The amplitude decreased and the direction changed slowly clockwise during the next 30 min, finally resulting in a tilt of 0.4 μrad towards the west No significant changes occurred in the tilt record at BVL (resolution 10^{-2} μrad) during this time.

A second creep sequence, on October 3, 1974 (Fig. 2*b*), is more important since it was observed on the four creepmeters MRW, MRB, MRC, and XMR1 along the fault from the south and to the north-west of MEL. The first onset of creep occurred at 0812 at MRC Almost simultaneous offsets occurred next at MRW and MRB at 0815 followed by XMR1 at 0837. The tilt changed at MEL between 0800 and 0815 by 0.52 μrad in a direction S72°W, and during the next hour this amplitude decreased and the direction changed slightly clockwise until reaching a final tilt offset value of 0.36 μrad in a westerly direction. The tilt records at BVL were again unchanged.

The creep sequence on April 14, 1975 (Fig 2*c*) was recorded on MRR at 0805, followed by MRC at 0821 and on XMR1 at 0826. Only the east–west tilt component was operating at MEL during this time This record shows a tilt change of 0.25 μrad between 0800 and 0807 similar to that observed for the earlier creep sequences.

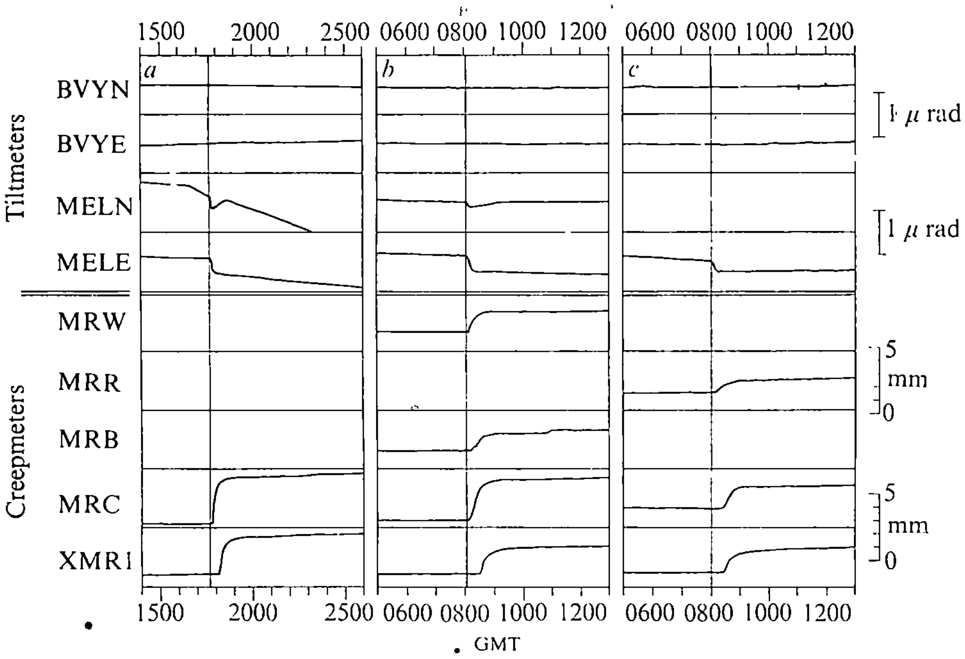

Fig. 2 Three examples of approximately 6 h of simultaneous digitised records from creepmeters XMR1, MRC, MRB, MRR and MRW and tiltmeters BVL and MEL during creep events on: *a*, July 11, 1974, *b*, October 3, 1974; *c*, April 14, 1975. BVLN, MELN, and BVLE, MELE are the north and east components, respectively Absence of data indicates an instrument malfunction

For the next pair of instruments to the north, creepmeter XFL1 and tiltmeter SAS with a 2-km separation, five creep sequences have occurred since June, 1973 when simultaneous recording began. Tiltmeter SAS is 1.2 km from the fault. At that distance, as for BVL, no clear relationship between the creep events and either short or intermediate term tilts has been found. This is true also for the northernmost instrument pair considered, creepmeter XPR1 and the tiltmeter LIB, separated by 3.4 km with LIB 1.5 km from the fault.

Creep-related tilts seem, therefore, to be rapidly attenuated as a function of distance from the fault. At Melendy Ranch, in particular, a displacement of a few millimetres on the fault that results in a tilt of 0.5 μrad at 370 m and less than 0.01 μrad at 1,500 m implies at least inverse cube attenuation with distance. This may be expected from either a small scale or shallow source or a slightly decreased rigidity in or near the fault zone. The latter possibility seems most likely since the surface displacements extend at least 1.2 km along the fault trace for the Melendy observations and seem in other cases to extend to several tens of kilometres[6]. This would also explain why a search for interrelationship between short period tilt changes on adjacent tiltmeters has so far been inconclusive.

The tilt signals for all three events are similar and therefore indicate a similar source process. The residual west tilt observed at MEL for the creep events, and the general systematic later arrivals and lower amplitudes of these and other creep events between MRC and XMR[8,9], indicate that the slip amplitude and/or the propagation velocity probably vary as the events propagate. Even with decreasing propagation velocity and slip amplitude to the north, however, the form of the tilt and the nearly simultaneous onset times for tilt and creep during the October 3 event (Fig. 2*b*) are difficult to fit with a single simple model of horizontal slip propagating northwards. The introduction of a vertical slip component (south-western side up) over a limited extent of the fault with a local maximum between MRB and MRC, together with horizontal slip, can generate time histories similar to those observed. The accumulated vertical displacement necessary as a consequence of this model is indicated by the presence of a north-easterly-facing scarp along the fault trace from MRW to MRC, with increasing scarp height to the north-west (the point of maximum development is about 3.5 m between MRB and MCR).

Most records of other creep events at Melendy Ranch display regular patterns of onset times[8,9] although variations similar to those for the October 3 and April 14 events occur. Simple slip models, such as indicated above, are not always compatible with the detailed onset times and the creep amplitudes. It is possible that the exact onset times of creep, and perhaps also the amplitudes, result from, or are modified by, the particular local failure conditions of the surface material. More examples on a supplemented tilt and strain array in this area should clarify many of these details and make a rigorous inversion possible.

We thank Bill Stuart and Wayne Thatcher for critically reading the manuscript. A more complete discussion will be held elsewhere.

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Received October 27, 1975, accepted March 3, 1976

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Compression and crustal shortening in Andean-type orogenesis

CONTROVERSY exists as to the role of crustal compression and shortening in the formation of the easterly-directed thrust system in eastern portions of the North American Cordilleran orogen[1,2]. In fact, the controversy can be extended to the general issue of the presence or absence of crustal shortening in any major phase of the development of that orogen. Opponents of crustal shortening have suggested that most fold and thrust structures of the Cordilleran orogen can be explained by gravity-induced stresses[3,4].

Tectonic analogies between the modern central Andes and the US Cordillera of Mesozoic and earliest Tertiary age have been discussed by Hamilton[5,6], James[7], and others. The Andes, a chain characterised by voluminous magmatic activity, are bounded on the west by the Peru–Chile trench, a zone of ongoing plate convergence, and on the east by an active fold and thrust belt which separates orogen from craton. Rates of subduction are of the order of 7–12 cm yr^{-1} (ref. 8), and can reasonably be extrapolated back at least into the late Miocene on the basis of marine magnetic anomaly studies[9]. Since Middle Miocene time the central Andes, including Peru, have experienced intensive igneous activity and accompanying orogenesis[10]. Folds, reverse faults and thrust faults of eastward vergence have developed along the eastern margin of the Andes in the Sub-Andean zone (Fig. 1). The Andes were lifted up to their present height during this late Cainozoic episode of orogenesis.

Stauder[11] in a study of seismicity in the Peruvian Andes, has concluded that the Nazca Plate is thrusting under the South American plate beneath central and northern Peru along a surface of shallow (10–15°) dip. Of particular importance to an understanding of Andean orogenesis is Stauder's additional conclusion that the leading (western) edge of the South American plate is under east–west horizontal compression for a distance of approximately 700 km east of the Peru Trench (Fig. 1). Numerous hypocentres are present in that plate at depths of 1–90 km and for distances up to 700 km east of the trench. Most lie at distances from the trench of 200 to 600 km, depending on latitude. Focal mechanisms for 9 or 10 of these intraplate earthquakes yield horizontal compressive stress axes oriented approximately east–west. Three solutions indicate predominantly strike-slip faulting and six indicate reverse dip-slip faulting.

Middle Miocene to Recent central Andean structural and magmatic features can best be explained by the effects of convergence of the Nazca oceanic plate toward and beneath the South American Plate. Magma genetically related to subduction of the Nazca Plate has intruded the leading edge of the South American lithosphere and led to its thermal weakening. Deformation in response to east–west horizontal compression is localised in the plate margin region of elevated temperature where the lithosphere yields variably by fracture and flow. Compressive thickening of the leading edge can account for the uplift of the Andean Chain. The Sub-Andean fold and thrust belt thus marks the present eastward limit of intraplate deformation resulting from subduction of the Nazca Plate. Gravity may have

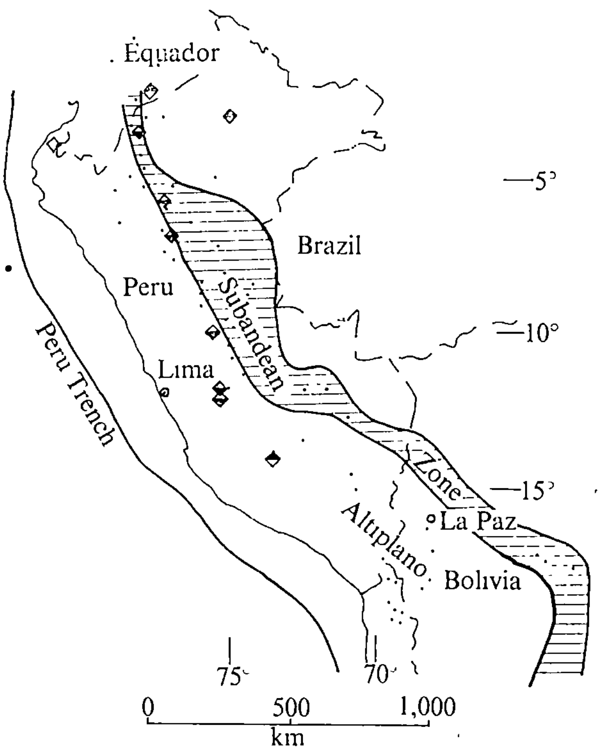

Fig. 1 Epicentres of shallow focus (1–70 km) earthquakes in the central and eastern part of the Peruvian sector of the Andes for 1961–68. Diamonds, earthquakes analysed by Stauder[11]; diamonds with blackened upper half, strike-slip solution; diamonds with blackened lower half, reverse fault solution; open diamonds, solution for north–south compressive stress and reverse faulting.

a minor role in the tectonic development of the Andean Orogen, but it is secondary relative to lithospheric compression and shortening.

The geological history of the Mesozoic and early Cainozoic Cordilleran orogenic belt of western North America suggests it developed in a structural setting much like that of the modern Andes. Key questions about the origin of the Cordilleran belt concern the role of horizontal compression in crustal deformation, particularly in the formation of the foreland or marginal fold and thrust belt which, before Tertiary basin-range extension, lay up to 800 km east of the convergent plate margin. Stauder[11] has concluded that the leading edge of the South American Plate beneath the Andes is under horizontal east–west compression for a distance at least 700 km east of the Peru Trench. His data demonstrate that crustal compression and shortening are possible in an Andean structural setting far from the offshore plate boundary, and support a compressive origin for older Cordilleran structures analogous to those of the central Andes.

This work was supported by the NSF.

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Caledonian magmatic activity in south-eastern Greenland

THE position of the Caledonian front in eastern Greenland is important[1,2] in any reconstruction of the pre-drift North Atlantic (see ref. 3). No evidence of any Caledonian activity has yet been found south of Scoresby Sund, however, where the Caledonian Front, exposed in Gåseland[4], is seen to pass under the thick plateau basalts of Kong Christian den IX's Land[5]. South of the basalts, only Precambrian ages have been obtained[2]. We report here on a complex of Caledonian age in the Kangerdlugssuaq district, which lies about 300 km to the south of the Gåseland exposures. Unknown Caledonian events may be recorded in basement rocks of the areas, and may be uncovered by more detailed investigation. Moreover, petrological similarities between the new complex and the alkaline suite found in Assynt, north-western Scotland, may provide a further link in the North Atlantic jigsaw puzzle.

We have had access to specimens from the Batbjerg Intrusion at the head of Kangerdlugssuaq and have published a description of leucite from this locality, along with a map showing its location and approximate extent[6]. The rocks were believed to be Tertiary, in common with those of the nearby Gardiners Plateau Intrusion (which it resembles in many aspects)[7] and other plutonic rocks of the area[5]. It was not until a visit in the summer of 1975 by a combined expedition from the Universities of Copenhagen and Toronto that many of the rocks in the Batbjerg Intrusion were found to be deformed and thus unlike the Tertiary rocks of the district. The impression that this complex is different from the nearby Tertiary rocks is confirmed by the new radiometric ages of around 440 Myr (Table 1). The dated rocks were selected as having primary magmatic textures, and we believe that the ages indicate crystallisation from a magma at the time of the Caledonian Orogeny.

In Scotland, alkaline rocks of a similar age[8] occur in the Assynt district. In particular, the Loch Borolan laccolith[9] is a layered complex of melanite pyroxenites, nepheline syenites and nordmarkitic syenites. Characteristic minerals are melanite garnet, acmitic diopside, green 'biotite', and rounded aggregates, interpreted as pseudoleucites.

Preliminary examination of the Batbjerg rocks shows that the bulk of the complex is composed of pyroxenites containing acmitic diopsides (an analysis has been presented in ref. 6), olivine, mica (sometimes green) and apatite. Other rocks include folded and sheared dunites, melteigitic rocks containing leucite and melanite, and a net-veined complex of syenitic dykes, some undersaturated, some granitic, cutting the pyroxenites. Many volcanic necks with associated cone sheets and ring dykes cut the surrounding gneisses, but they show extensive uralitisation of the pyroxenes and may be foliated. Masses of metamorphosed limestone (some with crinoid fragments) and quartzites occur along the contact between the pyroxenites and the gneisses They are interpreted as screens of country rock which have sunk from above.

It is clear that, superficially at least, there are many petrological similarities between Batbjerg and Loch Borolan. A speculative possibility is that the calcareous sediments at Batbjerg are equivalent to the Durness limestone and quartzites at Assynt, which had such a prominent role in Shand's now unpopular hypothesis for the development of the undersaturated rocks, both at Borolan and in

Table 1 Radiometric and petrographic data for the Batbjerg Complex, Kangerdlugssuaq

a Rb/Sr data

Sample	$^{87}Rb/^{86}Sr$*	$^{87}Sr/^{86}Sr$†
NM 3206/5 Diopside	0.00303 ± 0.00002	0.7046 ± 0.0003 (unspiked)
NM 3209 Phlogopite	28.4 ± 0.2	0.8819 ± 0.0006 (spiked)
NM 3206/5 Phlogopite	56.6 ± 0.4	1.0494 ± 0.0006 (spiked)

b K/Ar data

NM 3209	Phlogopite	^{40}Ar rad (standard cm^3 g^{-1} × 10^{-4})	1.4712	(mean of 2 determinations)
		^{40}Ar rad (%)	98.4	(mean of 2 determinations)
		K_2O (%)	9.54	(mean of 6 determinations by atomic absorption)
		Age (Myr)	446 ± 13	

c Petrographic notes

NM 3209	Dark pegmatitic pyroxenite with abundant brown phlogopite
NM 3206/5	Bright green, friable pyroxenite with about 10% pale greenish phlogopite

Both rocks are free from deformational fabrics and felsic minerals.

* Errors on $^{87}Rb/^{86}Sr$ ratios determined from 10 duplicates.

† Errors on $^{87}Sr/^{86}Sr$ ratios determined from 19 unspiked duplicates and 10 spiked duplicates.

Using $\lambda_{Rb} = 1.39 \times 10^{-11}$ the York-1 model provides an age of 443 ± 14 Myr with an initial $^{87}Sr/^{86}Sr$ ratio of 0.7046 ± 0.0016 (ref. 2). Standard errors include a 'Student's *t*' multiplier based on results for 10 duplicates.

other parts of the world[10]. Detailed petrological and mineralogical studies are at present being carried out on the Batbjerg rocks and will be published elsewhere. They should allow a more reliable comparison between the two intrusions. The gneisses surrounding the Batbjerg Intrusion are cut by an immense number of volcanic vents and associated dykes and cone sheets. The vents range in size from a few metres to perhaps half a kilometre in diameter and represent a further point of similarity to the Scottish and Irish Caledonides, where similar volcanic bodies, assigned to the appinite suite, are widespread[11,12]. Like the appinites, the Batbjerg vents are rich in hornblende and were emplaced by highly explosive, volatile-rich magmas.

An important question to be asked is, how widespread is Caledonian activity in the Kangerdlugssuaq district and further to the south? Existing radiometric ages from the gneissic basement of the area, which include K/Ar, $^{40}Ar/^{39}Ar$, Rb/Sr mineral and whole rock ages[2,13,14], give ages ranging from ~ 1,700 to 2,700 Myr. Sampling has not, however, been carried out with radiometric dating in mind, and no systematic mapping of the basement has yet been made. It is clear that a great deal more detailed work of this nature must be undertaken before the history of the basement can be unravelled with any certainty. In recent years it has become clear that old ages frequently occur in younger fold belts as evidenced by, for example, the 3,000 Myr isochron reported for samples from within the Caledonides of the Scoresby Sund district[15].

It is tempting to speculate that the close spatial association of similar rock types with widely differing ages is not coincidental. Thus, the Batbjerg Intrusion was originally regarded as Tertiary[6] largely because of its petrological similarity to the Gardiners Plateau Intrusion which cuts Tertiary plateau basalts and is not more than 10 km distant. The Tertiary age of the Gardiner Intrusion is confirmed by an unpublished fission track age of 54.8 ± 3.4 Myr (A. G. W. Gleadow, personal communication). It comprises ultramafic rocks (dunites, pyroxenites and melilite rocks or uncompahgrites) with smaller amounts of nepheline syenites, urtites and related rocks. As such it falls into the general category of alkaline ultramafic complexes, to which the Batbjerg Intrusion is also assigned.

A possibility which should be explored, probably by means of isotopic studies, is that the origins of both intrusions are attributable to an alkali-enriched upper mantle which has underlain this area for several hundreds of millions of years, a possibility that has been suggested in other cases Thus, it is now becoming clear that many ultramafic nodules in lavas apparently preserve an isotopic record of earlier mantle events. For example, Burwell[16] has recently reported nodules from Australia which lie on an isochron of about 700 Myr. That date coincides with a period of plate convergence similar to that in the North Atlantic during Caledonian times.

Until this report of Caledonian rocks in the Kangerdlugssuaq district, there has been no basis whatsoever for showing the Caledonian front in eastern Greenland passing out into the Denmark Strait near the mouth of Kangerdlugssuaq Fjord, as has been done by Flinn[3]. Indeed, rocks from the only basement exposure to the east of this line had already been reported to have radiometric ages of over 2,000 Myr. In the light of these considerations, it must be concluded that the position of the Caledonian front in this area is unknown and cannot therefore be used for pre-drift reconstructions. The discovery of rocks of Caledonian age in the Kangerdlugssuaq district indicates, however, that preconceived ideas on the basement rocks of southern Greenland are not necessarily correct. It is clearly desirable to carry out further work on the basement in that region.

We thank Ole Larsen and D.C. Rex for age determinations (Table 1). The field work was supported by the Danish Natural Sciences' Research Council and the National Research Council of Canada. Preliminary samples were put at our disposal by the Northern Mining Company, Copenhagen.

J. D. Friderichsen and A. K. Higgins of the Geological Survey of Greenland have offered valuable comments.

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Na, P, Ti and coordination of Si in garnet from peridotite and eclogite xenoliths

THEORY and experiment show that some cations are surrounded by extra oxygen anions at elevated pressure: for example, Si changes from 4-coordination in quartz and coesite to 6-coordination in stishovite. Sobolev and Lavrent'ev[1] suggested that substitution of Na in garnet derived from the mantle is coupled with 6-coordinated Si according to the equation $Ca^{VIII}+Al^{VI}\rightarrow Na^{VIII}+Si^{VI}$. We show here from sensitive electron-microprobe analyses that Na in garnets from ultramafic and eclogite xenoliths can be explained by coupled substitutions with Ti and P without invoking 6-coordinated Si.

Table 1 records mean values for simultaneous electron-microprobe analyses of selected garnets using a strong electron beam and long counting times. Backgrounds were carefully estimated at several wavelengths above and below the peak, and the standard deviation of individual analyses is 15–25 p.p.m. Cracks and surface blemishes were avoided during analysis with a 70-μm diameter spot, and 20 out of 21 garnets were found to be homogeneous from analyses at 3–6 places. Standards were $Di_{85}Jd_{15}$ glass (Na), A408 apatite (P) and Corning V glass (Ti). The correction factors for Na and Ti were trivial and were ignored, but correction factors for P were applied from the GLAB program (L. W. Finger, personal communication) using major-element concentrations from solid state detector and spectrometer analyses with a 2-μm diameter electron beam.

The concentration ranges for Na_2O (100–1,400 p.p.m. by weight) and TiO_2 (210–7,800) do not exceed those given previously for Na in garnets associated with diamond[1] and Ti in garnets from peridotite and eclogite xenoliths[2]. The range for P_2O_5 (160–940) is within that for garnets synthesised from basaltic melts at 1.8–4.5 GPa (18–45 kbar) pressure[3]. No previous analyses of P_2O_5 in mantle-derived garnets have been reported.

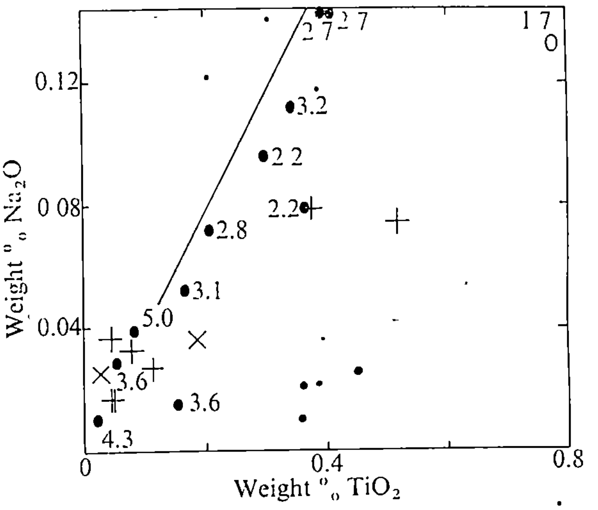

Fig. 1 Weight % Na_2O against TiO_2 in mantle-derived garnets: +, garnet–lherzolite; ×, other ultramafic rock; ●, eclogite; ○, diopside megacryst with garnet and sulphide inclusions. The numbers are K_D as defined in Table 1 for the eclogites. The straight line represents equal atomic concentrations of Na and Ti.

The Na concentration generally increases with the Ti concentration (Fig. 1), and in all but six garnets, atomic Ti exceeds atomic Na. A rough correlation exists also between Na and P, but the spread is well outside the analytical error band. In all but one garnet atomic Na exceeds atomic P.

Because atomic Na is always less than atomic (Ti+P), there is no need to invoke 6-coordinated Si (Fig. 2). Substitutions of the type $Ca^{VIII}+Si^{IV}\rightarrow Na^{VIII}+P^{IV}$ and $Ca^{VIII}+Al^{VI}\rightarrow Na^{VIII}+Ti^{VI}$ retain the normal valence and coordination number of Si, Ti and P. Thilo[4] reported garnet structure for $Na_3Al_2P_3O_{12}$ and $NaCa_2Mg_2P_3O_{12}$ compositions. Ringwood and Lovering[5] suggested that $(CaNa_2)Ti_2Si_3O_{12}$ and $(Ca_2Na)(AlTi)Si_3O_{12}$ are the probable Na-bearing components in garnet synthesised from a clinopyroxene–ilmenite nodule at pressure > 10 GPa. Although 6-coordinated Si may occur in the lower mantle as stishovite (see ref. 6), there is no evidence for its existence in minerals ejected to the Earth's surface from the upper mantle.

Table 1 Analyses of mantle-derived garnet

Label	Host rock*	Location	Weight TiO_2 (p.p.m.)	P_2O_5 (p.p.m.)	Na_2O (p.p.m.)	K_D
740	garnet–lherzolite	Lashaine	460	200	170	2.1
794	garnet–lherzolite	Lashaine	430	320	170	2.4
749	garnet–wehrlite	Lashaine	1,900	300	380	2.3
1143B	garnet–lherzolite	Bultfontein	790	280	330	2.8
1149	garnet–lherzolite	Bultfontein	460	690	370	2.5
1156	garnet–lherzolite	Bultfontein	5,200	360	750	2.2
1359	garnet–lherzolite	Matsoku	3,800	500	790	2.2
1755	garnet–pyroxenite	Klipfontein	290	390	250	3.6
1870/2	garnet–lherzolite	Letseng-le-Terai	1,130	300	270	3.4
1178	eclogite	Roberts Victor	4,100	500	1,400	2.7
1187	eclogite	Roberts Victor	3,000	530	950	2.2
1188	eclogite	Roberts Victor	820	230	390	5.0
1190	eclogite	Roberts Victor	3,400	800	1,120	3.2
1191	eclogite	Roberts Victor	1,700	250	520	3.1
1692	eclogite	Roberts Victor	4,000	520	1,400	2.7
1780/1	eclogite	Newlands	3,600	710	790	2.2
1780/2	eclogite	Newlands	2,100	460	720	2.8
1829/1	eclogite	Bobbejaan	550	910	280	3.6
1829/2	eclogite	Bobbejaan	210	160	100	4.3
1934	eclogite	Vissuri	1,600	260	150	3.6
73–267	clinopyroxene megacryst	Monastery	7,800	940	1,300	1.7

K_D = (FeO/MgO)gt/(FeO/MgO)cpx.
* All garnet lherzolite, pyroxenite and wehrlite host rocks exhibit granular texture.

The validity of geothermometers and geobarometers involving pyroxene and garnet is controversial, but the parameter $K_D = (FeO/MgO)gt/(FeO/MgO)cpx$ has been used to classify eclogites on a probable depth basis (see ref. 7), and has been shown from laboratory syntheses[8] to decrease with increasing temperature and decreasing pressure. There is a weak tendency for Na, Ti and P in the garnets from eclogites to increase as K_D decreases (for example in Fig. 1), which might suggest that substitution of these minor elements in garnet increases with the depth of formation. Elsewhere we show that the correlation between K_D and Na_{gt}/Na_{cpx} for lherzolites and eclogites is good. Much further work is needed on the factors which control the substitution of minor and trace elements in mantle minerals, especially because partial melting should lead to strong fractionation.

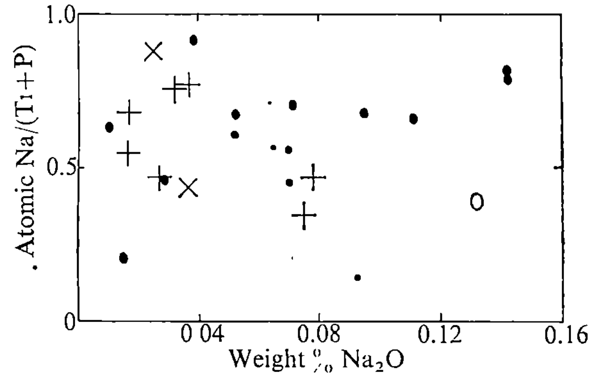

Fig. 2. Weight % Na_2O against atomic Na/(Ti+P). See Fig. 1 for symbols.

We thank O. Draughn, T. Solberg and I. M. Steele for help and the NSF and Materials Research Laboratory for support.

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Received March 4, accepted March 18, 1976.

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Tree growth and climatic cycles in the rain shadow of the Grampian mountains

TREE-RING widths have been widely studied as a guide to historical meteorology[1,2], the assumption being that fluctuations superimposed on the smooth progression of change with age are climate induced. Several authors[3,4] have even identified cyclical patterns in tree growth, some of which they have tentatively linked to sunspot activity, but generally the precise climatic parameter that may be operating remains unspecified. In Scandinavia, ring widths seem to fluctuate with summer temperature[5,6], although precipitation during early summer may also be influential[7]. In Scotland, on the other hand, Schove and Frewer[8] were unable to relate any meteorological character to the growth pattern of native Scots pine (*Pinus sylvestris* L.) on Speyside. Here we report, however, that examination of the results from two long term fertiliser experiments in mature Scots pine at Alltcailleach forest (National Grid Reference NO 3596), on upper Deeside, has shown that growth exhibits several regular oscillations each of which can be related to a particular climatic cycle. Furthermore, it would seem that some of these climatic cycles may be limited to low rainfall areas, such as the rain shadow of the Grampian mountains.

Table 1 Correlations between ring width deviation, May rain during the same year, annual temperature during the same year and rain during May plus June of the previous year

	D	$M^{0.1}$	T	MJ
D	—	0.453	0.379	0.423
$M^{0.1}$	$P < 0.01$	—	−0.095	0.176
T	$P < 0.05$	NS	—	0.220
MJ	$P < 0.01$	NS	NS	—

The trees, planted in 1883 on a freely drained humus iron podzol developed on a terrace of the river Dee, remained virtually unthinned until 1934. Since then numerous light thinnings have been carried out at no fixed interval. In late 1972 increment cores were drilled from opposite sides of 32 untreated trees and the width of each of the 40 outermost rings measured to the nearest 0.01 mm. Meteorological data used is that published[9] for Balmoral (~10 km due west and upstream) where the mean annual rainfall and daily temperature are 830 mm and 6.5 °C, respectively

During the 40 yr, 1933–1972, average ring widths declined linearly ($P<0.05$) and the deviations have been taken from the fitted line. Preliminary inspection of a segment of the data suggested that correlations with winter climate were very unlikely. Ring width deviations, therefore, were tested for correlations with rainfall amounts and mean temperature recorded over the full year and during the six summer months April–September, individually and in all possible combinations. Only three climatic parameters were significantly and independently related to ring width deviations (Table 1), two of which—annual temperature and the previous year's May–June rainfall—were linearly related, whereas the positive response per unit of May rainfall became less pronounced as the amount increased. These three terms are all significant when included in the following multiple regression, the multiple correlation coefficient for which is 0.67: $D = -2.8765 + (1.2339 \pm 0.3555)M^{0.1} + (0.1371 \pm 0.0485)T + (0.0012 \pm 0.0006)MJ$ where D is the ring width deviation (mm), M is May rain of the same year (mm; M 62.5, s.d. 30.4), T is annual temperature of the same year (°C; T 6.48, s.d. 0.55) and MJ is the total rain during May–June of the previous year (mm; MJ 113.1, s.d. 45.0).

Ring width deviations and these three climatic parameters were then subjected to spectral analysis followed by repeated curve fitting about the indicated harmonics. Three significant oscillations, and one just short of significant, were found in

Table 2 Climatic oscillations associated with the oscillations in ring width deviation shown in Fig. 1

	Period (yr)	Significance of fit	Amplitude ±	Mean	Lag between climate and ring width (yr)
Annual temperature	42.0	$P < 0.01$	0.34 °C	6.48 °C	None
May rain	23.0	$P < 0.01$	17.9 mm	62.5 mm	0.6
May rain	11.9	$P < 0.01$	21.0 mm	62.5 mm	None
June rain	4.44	$P < 0.05$	15.1 mm	50.9 mm	1 2

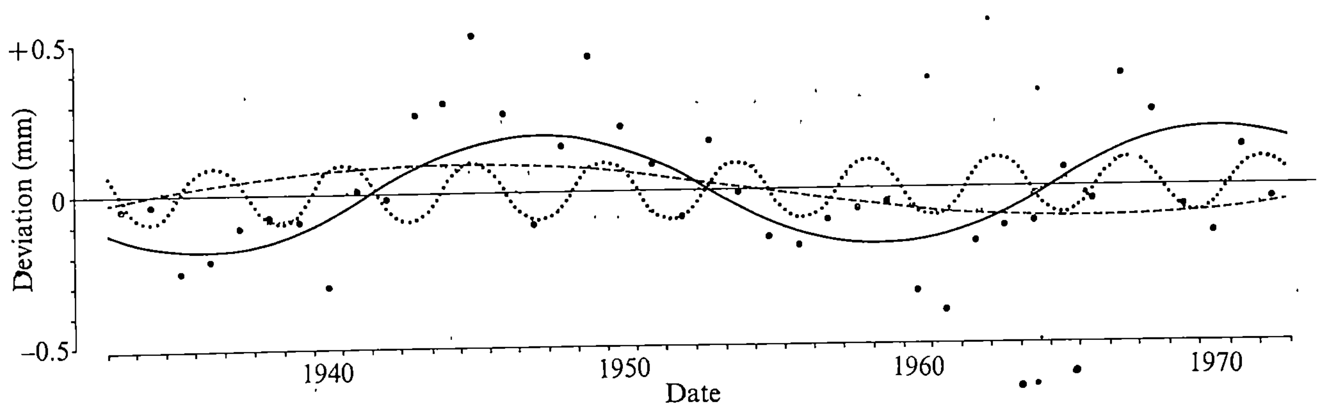

Fig. 1 Observed values of ring width deviation (●) and fitted oscillations; – – – – 42.0-yr period with amplitude 0.094 mm ($P < 0.05$), —— 23.0-yr period with amplitude 0.184 mm ($P < 0.001$), ·········· 4.44-yr period with amplitude 0.093 mm ($P < 0.05$) and a barely significant oscillation (fine dotted line) of 11.9 yr with amplitude 0.091 mm ($P < 0.1$).

Table 3 Occurrence of the four climatic oscillations associated with tree growth at Alltailleach forest at meteorological stations both to the west and east of the Grampian mountains

Station	Latitude	Longitude	Parameter and period (yr)			
			Annual temperature 42.0	May rain 23.0	May rain 11.9	June rain 4.44
Western						
Stornoway	58°13′N	06°20′W	$P < 0.01$	NS	NS	NS
Rothesay	55°50′N	05°04′W	NS	NS	NS	NS
Fort Augustus	57°08′N	04°40′W	$P < 0.05$	NS	NS	NS
Eastern						
Nairn	57°35′N	03°54′W	$P < 0.05$	NS	$P < 0.05$	NS
Braemar	57°00′N	03°24′W	NS	$P < 0.001$	$P < 0.05$	($P < 0.1$)
Balmoral	57°02′N	03°12′W	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$
Aberdeen	57°08′N	02°08′W	$P < 0.05$	$P < 0.01$	$P < 0.001$	NS

the ring width deviations (Fig. 1); for each there was a matching oscillation in climate (Table 2) and there were no significant oscillations in these climatic parameters that were not reflected in the ring width deviations.

The absence of any previous mention of these relationships with tree growth in the British Isles, and the predominant influence of spring drought, promoted the idea that the climatic oscillations in Table 2 may be less pronounced, or may not occur, outside the rain shadow of the Grampian mountains. Accordingly the same climatic oscillations were looked for in three stations to the south and west and four stations to the north and east of the mountains (Table 3), the prevailing winds being southwesterly. The effect of the mountains on the oscillations in rainfall is quite striking, indeed the short term oscillation in June rain could only be detected at Balmoral and, less certainly, Braemar, two stations well up the Dee valley and tucked in behind the main mountain mass. These oscillations, therefore, may reflect variations in the strength of the westerlies. Further support for this suggestion can be found in a strikingly close similarity between the ring width pattern found at Alltcailleach and patterns published recently[10] for pine growing within the rain shadow of the Norwegian coastal mountains

Experimental interest in this tree stand was originally prompted by the belief of local Forestry Commission management staff that tree health and growth were declining severely It now seems that the decline from a peak positive deviation in 1945 to a large negative deviation in 1960 (Fig. 1) is primarily a reflection of the 23-yr cycle in May rain with the addition of the longer term temperature cycle. Irrespective of the experimental fertiliser application given in 1960, tree growth improved over much of the next decade but as the temperature was at the bottom of its cycle the optimum conditions of 1945 did not recur.

The number and length of these climatic cycles, coupled with the geographical variability in both their occurrence and their significance for tree growth, must counsel caution over recent attempts to explain patterns and trends of tree growth in terms of atmospheric pollution.

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Energy expenditure in small children of obese and non-obese parents

Several recent studies have suggested the possibility of identifying some of the factors in infancy which contribute to the development of obesity in later life. For example, the abnormally rapid gain in weight of those infants who tend to become overweight later may be associated either with some genetic factor or with early overfeeding[1,2]. It is, however, highly probable that a child born to obese parents will itself become obese[3]. The main aim of this study was to see if it was possible to identify a difference in energy expenditure between children of 'normal' and 'obese' parents.

The energy intake and expenditure have been estimated in two groups of 4–5-yr-old children distinguished, not on the basis of their own weight, but according to the classification of their parents as being obese or non-obese. We found a difference which could be the result of either genetic or environmental factors. At this early age none of the children was significantly overweight for age or height, and the interest of this approach lies in the fact that any such differences observed may relate to factors contributing to obesity in later life.

Intake and expenditure measurements were made on 17 children and in a further three expenditure only was estimated. Height, weight and skinfold thickness were measured in all subjects. Body density was calculated from the regression equation of Brook[4] and body fat from the equation given by Siri[5]. Lean body mass (LBM) was obtained by subtracting the fat content from the total body weight. Apart from age and apparent good health, the main criterion of selection of subjects was the willingness of the parents and children to take part. The children were divided into two groups, those with one parent whose adult weight had at some time been more than 20% above desirable weight for height[6] (group O), and the remainder, neither of whose parents had ever been overweight (group N).

Daily energy expenditure was estimated from the integrated pulse rate for the day, based on the relationship between oxygen consumption and pulse rate. To establish this relationship, or to 'calibrate' the children, simultaneous measurements of oxygen consumption and heart rate were made on each child while he or she was lying down at rest and again while pedalling a stationary tricycle. Oxygen consumption was measured with a continuous air flow diaferometer, thereby eliminating the need for a face mask. Wherever possible more than two points on the calibration line were obtained. Integrated heart rate was then recorded on a SAMI (Socially Acceptable Monitoring Instrument) for 4 to 7 days and nights while the child went about his or her normal activities.

Energy intake was measured by analysing duplicate portions of all the food consumed by each child over a period of 5–7 d and the energy content of these portions estimated by ballistic bomb calorimetry[7]. The consumption of drinks was recorded separately and the energy content calculated from food tables[8,9].

Table 1 shows that although the two groups do not differ in body size, the estimates of energy intake and expenditure, including expenditure at rest, are significantly lower in group O than in group N. The degree of significance is greater when the results are expressed per kg body weight. The correlation coefficients of body weight, body fat and LBM with energy intake, expenditure and resting rate of expenditure have been calculated for all subjects. The only significant correlations are those of resting oxygen consumption with body weight (r +0.58) and LBM (r +0.64). The results show therefore that the resting oxygen consumption is affected by both body weight and by having one obese parent. Analysis of covariance shows that having an obese parent is highly significant over and above the effect of body weight, the latter tending if anything to mask the effect of the former.

The most critical questions arising from this study are whether the differences between the two groups result from metabolic or environmental factors or both, and whether these in turn are the result of genetic or acquired differences.

Energy is required for maintenance, physical activity and growth, the last accounting for only about 2% of the total expenditure[10]. The major portion is used for maintaining and resynthesising body tissue, to cover the heat increment of feeding and the minimum level of muscular activity needed for sustaining body functions. Energy expended while a subject is relaxed and inactive, as were the children in this study when they were at rest, would be expected to be similar to their maintenance requirement. The mean resting energy expenditure of all the children calculated on a 24 h basis was 1,110± 190 kcalorie.

The daily requirement of energy for maintenance has been estimated[10] to be ~ 105 kcalorie per kg$^{0.75}$ or 1.5 × basal metabolic rate. A similar figure was obtained by Spady[11] based on a study of energy balance in children recovering from malnutrition. For the children in this study 105 kcalorie per kg$^{0.75}$ averages 970 kcalorie. This is close to the average resting expenditure of the two groups (1,110 kcalorie). It seems reasonable, therefore, to take the difference between the observed total expenditure and resting expenditure as an estimate of the energy cost of voluntary physical activity. On this basis the energy expended on physical activity by the children in group O (190 kcalorie) was only half that expended by the children in group N (371 kcalorie).

It is of note that in a study carried out in the 1930s (ref. 12), the mean energy intake of 4–5-yr-old children was found to be 1,770 kcalorie. The maintenance requirement calculated according to the FAO/WHO estimate[10] would have been about 970 kcalorie, leaving 800 kcalorie available for physical activity —2–4 times that of the children in the present study. We know of

Table 1 Mean daily energy intake and expenditure

	Differences between groups‡				
Body size	Group N	s.d.	Group O	s.d.	Difference
Height (cm)	111.3	7.4	110.7	7.5	*
Weight (kg)	19.08	3.53	19.49	3.46	*
Body fat (%)	14.3	5.2	16.8	3.7	*
LBM (kg)	16.2	2.24	17.0	1.71	*
Standard weight for height (%)	99 9	9.5	100.8	6.0	*
Energy measurements (kcalorie)					
Daily intake	1,433	188	1,115	300	$P<0.01$†
Intake per kg body weight	75	13	57	8	$P<0.01$†
Daily expenditure	1,508	352	1,174	297	$P<0.05$†
Expenditure per kg body weight	79	18	60	9	$P<0\ 02$†
Daily expenditure at rest	1,183	184	999	146	$P<0.05$†
Expenditure at rest per kg body weight	62	9	52	6	$P<0.02$†
Daily expenditure available for activity	371	306	190	173	$P<0.2$*
Groups combined					
Daily intake	1,325	263			
Daily expenditure	1,375	364			
Daily expenditure at resting rate	1,110	190			
Daily expenditure available for activity	265	266			

* Not significant.
† Significant.
‡ Number in group N = 12; number in group O = 8 (except for intake study when it was 5).

no evidence to suggest that basal metabolic rates of children have fallen dramatically over this time, and the conclusion that the decrease in intake is associated with changes in patterns of behaviour seems inevitable. These, in turn, may reflect changes in the attitudes of parents towards expected levels of activity and play of children. Other aspects of living such as television and domestic heating standards may also be contributory.

We found a difference between the energy expenditure of children of normal and obese parents which is of the same order (350 kcalorie d^{-1}) as the decline in average intakes over the past 40 yr. Although this decline is likely to be due to environmental changes affecting voluntary activity levels, the children of obese parents also have lower resting rates of energy expenditure, and it will be of great importance in future studies to determine if this is the result of behavioural or of inborn metabolic differences.

This work was supported by the MRC, Rank Hovis McDougall Ltd, and the Weight Watchers.

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Received December 8, 1975, accepted March 3, 1976.

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Molecular evidence for dual origin of mangabeys among Old World monkeys

THE morphological similarities among the various mangabey species have been uniformly judged by primate systematists to warrant their association in a single genus (*Cercocebus*) of Old World monkeys. These similarities are not, however, reflected in their macromolecules. We find that the two groups of mangabeys are, by Old World monkey standards, so different at the protein level as to force the conclusion that the genus is not a natural, that is, monophyletic, group.

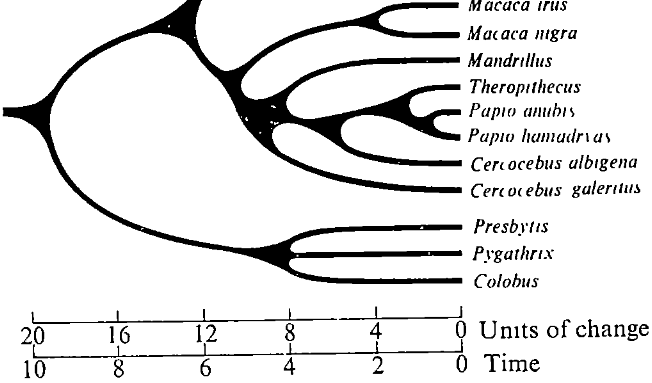
Molecular data have become an important, independent source of phylogenetic information. Though their application to specific problems in systematics has been limited, they have revealed a common origin for the New and Old World higher primates[1-3]; the monophyly of the pinnipeds among the arctoid carnivores (ref. 4 and paper presented at Society for Systematic Zoology meetings, 1975); the ursid nature of the giant panda[5,6], and the association of the tree frog *Hyla wrightorum* with *H. eximia* instead of *H. regilla*[7]. In each case, however, the problems were longstanding and the possible solutions available; the molecular data provided independent and objective ways of choosing among the possibilities. On the other hand, new sources of information can point out unsuspected situations, such as the strong probability that man is as closely related to the chimpanzee and gorilla as they are to each other (refs 1, 8–10 and an unpublished result).

The mangabey situation seems to be an example of this latter phenomenon, involving the probable misplacement of two species (*C. albigena* and *C. aterrimus*) among the Old World monkeys. We present here evidence that the two mangabey species groups—one containing *C. galeritus* and *C. torquatus* and the other *C. albigena* and *C. aterrimus*—may well be cladistically unrelated. We find our various sera representing the former species group (obtained as *C. fuliginosus* from the Davis Primate Center, and *C. galeritus* and *C. torquatus* from the Delta Primate Center) to be virtually indistinguishable both immunologically and electrophoretically. On the other hand, they are as distinct by these criteria from *C. albigena* and *C. aterrimus* as from any of the other 42-chromosome cercopithecine species (macaques and baboons). Finally, the *albigena–aterrimus* group seems to be an early offshoot of the baboon lineage, diverging from it some time after the separation of the *Mandrillus* (drill and mandrill) and *Macaca* lines and well before the *Papio–Theropithecus* radiation.

The Old World monkeys are divided into two subfamilies, Cercopithecinae and Colobinae, with the former containing the genera *Papio*, *Theropithecus*, *Cercocebus*, *Macaca* and *Cercopithecus*. These five contain some 35 species, and the strong evolutionary affinities among at least the first four are evidenced most strikingly by the ease with which viable intergeneric hybrids are produced[11,12]. With so complex a grouping of closely related forms and the absence of a detailed fossil record[13], we felt that a molecular study would prove useful in adding a new dimension to our systematic knowledge of the group, and the *albigena–aterrimus* finding is one striking result.

Antisera to numerous purified Old World monkey albumins and transferrins[14] were prepared in rabbits (Dutch belted, three or four per immunogen) according to published procedures[3,15] and pooled in reciprocal proportion to their microcomplement fixation (MC′F) titres. Differences among the various albumins and transferrins were measured using the MC′F procedure and are expressed as immunological distances[3]. Immunological distances are highly correlated with, and linearly proportional to, the number of amino acid sequence differences among the protein species being compared[16]. They are also additively apportionable along derived cladograms[2,3,17,18] and thus serve as reliable indicators of phylogenetic affinities among the taxa being compared. The data reported here were obtained using antisera to the albumins of *Papio cynocephalus*, *P. papio*, *Macaca irus*, *Cercopithecus*

Fig. 1 Albumin plus transferrin phylogeny of the Old World monkeys. This cladogram has been derived using procedures detailed in ref. 2. The complete data sets will appear in a comprehensive article on Old World monkey systematics. Immunological distances among the albumins and transferrins of the various species were obtained using the antisera listed in the text. The immunological distance separating any two forms was apportioned additively along the two lineages using antisera directed to the proteins of species outside the taxon involved. All such lineage pairs were then superimposed along common segments while minimising the differences among the experimental immunological distances and those derived from the cladogram.



aethiops and *Presbytis entellus*, and to the transferrins of *Theropithecus gelada*, *P. cynocephalus*, *P. papio*, *P. hamadryas*, *M. irus*, *M. nigra*, *M. speciosa*, *C. torquatus*, *C. patas*, *P. entellus* and *Colobus guereza*.

The detailed molecular systematics of the Old World monkeys will be presented elsewhere, but it is necessary to place the major lineages relative to one another before considering the position of the *albigena–aterrimus* group among them. The phylogeny is presented in Fig. 1 and its reliability can be assessed by comparing the immunological distances calculated from it and the experimental values. With each of the latter is associated an uncertainty deriving from non-reciprocity and experimental error, and these have appreciable significance given the very small distances involved in this study. The non-reciprocity of the input data matrix is ±1–2 units and the experimental error of each measurement is also between 1 and 2 units. Given this level of uncertainty or noise in the input data, the mean difference of 2 units between the elements of the input and output data half-matrices can only be taken to indicate that we would find essentially perfect additivity if we could deal with a set of data uncontaminated by immunological noise. But the observed noise level of 2 units should be remembered when assessing the reliability of the lineage placements in Fig. 1. We have attempted to indicate this visually by making the nodes areas rather than points. We then find the 42-chromosome Cercopithecinae (Papionini of Jolly[20,21]) forming a clade relative to *Cercopithecus*; within that clade *Theropithecus–Papio*, *Macaca*, *Mandrillus* and the *galeritus–torquatus* mangabeys form the major lineages.

The placement of the *albigena–aterrimus* lineage within this phylogenetic framework follows from a consideration of the data in Table 1 plus the albumin plus transferrin distances from *Papio* to: *Theropithecus*, 10 units; *Mandrillus*, 18; *Macaca*, 27; and *Cercopithecus*, 34. The distance between the two mangabey groups is 26 units, and the evolutionary significance of these observations is made evident when two colobines (*Presbytis* and *Colobus*) are used as reference species to assess the relative amounts of change in the two molecules along the lineages of interest. The colobine–*Papio* distance is 50 units; to the *galeritus–torquatus* mangabeys it is 51, and to the *albigena–aterrimus* mangabeys, 50. Thus in this case the albumins and transferrins have accumulated equal numbers of substitutions in the three lineages; therefore the fact that the two *albigena–aterrimus* molecules are more similar to their *Papio* counterparts than to those of the other mangabeys cannot be ascribed to a lack of change along either the *albigena–aterrimus* or *Papio* lineage. Thus we must conclude that the immunological data do not associate the two mangabey groups subsequent to the adaptive radiation of the Papionini and do indicate that the *albigena–aterrimus* lineage is probably an early offshoot of that leading to *Papio* and *Theropithecus*. This conclusion concerning the mangabeys is, of course, not the only interesting one from this study. The others will form subjects of future reports, but we note here the following. The various *Papio* species and *Theropithecus* are seen as very closely related at all levels surveyed here—albumin and transferrin immunology and plasma protein electrophoresis. Similarly the patas monkey, *Cercopithecus* (or *Erythrocebus*) *patas*, is found to be more closely related to the green monkey (*Cercopithecus aethiops*) than is any other *Cercopithecus* species. Finally the drill and mandrill (*Mandrillus*) form a lineage which is not specifically associated with *Papio–Theropithecus* (and, now, the *albigena–aterrimus* mangabeys).

In addition to the immunological studies just discussed, we have carried out some preliminary electrophoretic comparisons of the various sera used in this study. In the conditions used (7% acrylamide in a continuous 0.1 M Tris, 0.05 M glycine buffer), we typically see 20–25 serum protein bands staining with Coomassie blue. One can then use as a measure of similarity (*S*) between two samples simply the ratio of the number of bands with the same mobility in the two to the number of bands in the sample containing the smaller number.

Table 1 Albumin plus transferrin immunological distances between *Cercocebus* and other Old World monkeys

	C. galeritus	*C. albigena*
*C. galeritus**	0	26
C. albigena	26	0
T. gelada	25	16
Papio†	24	12
Mandrillus	16	12
Cercopithecus	20	30
Macaca‡	16	18
Colobinae§	51	50

*Antisera made to the albumin of a *C. galeritus* from the Delta Primate Center and to the transferrin of a *C. torquatus* from the Davis Primate Center fail to distinguish any of the various *galeritus–torquatus* individuals from each other

†Using antisera to the albumins of *P. papio* and *P. cynocephalus* and to the transferrins of *P. papio*, *P. cynocephalus*, *P. anubis*, *P. hamadrayas* and *P. ursinus*.

‡Using antisera to the albumin of *Macaca fascicularis* and to the transferrins of *M. fascicularis*, *M. nigra* and *M speciosa*.

§Using antisera to the albumin of *Presbytis entellus* and to the transferrins of *P. entellus* and *Colobus guereza*.

For intergeneric comparisons involving *Macaca*, *Papio*, *Cercocebus galeritus*, and *Mandrillus*, the observed *S* values are in the range 0.1–0.2. The *S* value between *P. cynocephalus* and each of the following is: *P. hamadryas*, 0.75; *T. gelada*, 0.6; and *C. albigena* or *aterrimus*, 0.35. Among the various *torquatus–galeritus* individuals in our collection, the *S* values are no less than 0.8; for the *albigena–aterrimus* comparison the value is 0.8; and between individuals in the two mangabey groups it is 0.15–0.2. Thus these preliminary electrophoretic data are consistent with the immunological picture.

Previous electrophoretic studies have detected marked differences in the mobilities of the α and β chains of *C. albigena* haemoglobin as compared with those of *C. galeritus*[21]. In addition, the peptide pattern of *C. albigena* haemoglobin is more like that of *Papio* than of *Macaca*[22]. This then completes a phylogenetic congruence among all the available molecular data relevant to mangabey systematics. We suggest that a DNA hybridisation study would provide useful additional information.

We emphasise that these conclusions concerning the positions of the two mangabey lineages should be regarded as suggestive working hypotheses to be tested in terms of the degrees to which they facilitate the interpretation of other bodies of comparative data bearing on cercopithecine evolution. One such test might begin with the observation that the mangabeys have sufficient morphological uniformity to have been placed consistently in a single genus—which is very significant in view of the tendency to splitting evident in Old World monkey taxonomy[11,23]. This morphological uniformity contrasts sharply with the molecular picture just presented. Such a contrast is no longer surprising in the context of the extensive recent documentation of the independence of rates of structural protein and organismal evolution[24–27], but its presence provides the opportunity to develop important insights into the evolution of the Cercopithecinae. If the contrast is real, then either mangabey morphology is primitive for the Papionini (baboons, macaques and mangabeys), or it is derived, in parallel or convergently, from the common ancestor of the group. This latter possibility is rendered unlikely by the fact that no one has yet seen mangabey morphology as derived[19,20,28,29]. Thus we are left with the former, leading to the important logical corollary that the larger derived Papionini—drill and mandrill, savanna baboons and gelada, and some large macaques such as *M. nigra* and *M. nemestrina*—simply represent slightly different ways of being a large Old World monkey starting from a mangabey-like morphological base.

One of the *albigena* samples (19354) was obtained from the Delta Regional Primate Center (DRPC) through Dr Charles Hill, and the other from eastern Zaire through Dr Urs Rahm. The two were immunologically indistinguishable. *C. aterrimus*

was provided by Dr Kurt Benirschke of the San Diego Zoo; *Papio* and *Theropithecus* by Dr J. Moor-Jankowski of LEMSIP; *Mandrillus*, *C. ascanius* and *C. guereza* by Dr William Mottram of San Francisco Zoo; *C. galeritus* (061-6979, 6984, 8512, 9093), *C. torquatus* (062-6992, 7004, 7191), *C. aethiops* and *C. patas* by DRPC; *C. torquatus* (CFU-2, 14, 26, 28) and *P. entellus* by Davis Regional Primate Center and *Macaca* by Dr Marjorie LaSalle of the Oregon Regional Primate Center. All work was done in the laboratory of Dr Allan C. Wilson of the University of California, Berkeley. We thank him for facilities and helpful discussion, together with S. L. Washburn and W. Malmi. Technical assistance was provided by Anne Hill and Peter Hornbeck and support through grants from the US National Science Foundation to A.C.W. and V.M.S. and a predoctoral fellowship to J.E.C.

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Magnesium emitted by snails alters swimming behaviour of *Schistosoma mansoni* miracidia

Schistosoma mansoni is a parasitic flatworm transmitted by snails which infects millions of people in tropical countries with poor sanitation, where new irrigation schemes, population growth and continued poverty have increased the prevalence of infection. Larval worms, emerging from freshwater snails, penetrate human skin and mature in intestinal veins where they lay eggs. Many eggs remain in and damage tissues while others pass out in excreta and hatch in water, releasing embryos called miracidia These short lived, multicellular, ciliated, free-swimming miracidia must find, penetrate and reproduce within certain snails whence emerge the larvae infective for man. Snails emit substances ("miraxones") which alter the swimming behaviour of miracidia and may help them to locate and attack the snails[1-3]. Miracidial behaviour is reportedly affected by snail-elaborated amino acids[4,5] and by various other agents, including fatty acids[6,7]. We now report that magnesium appears in water in which uninfected *Biomphalaria glabrata* (a snail host of *Schistosoma mansoni*) is confined, and that magnesium ions alter miracidial swimming patterns.

Methods for rearing Puerto Rican (PR-2′) *B glabrata* and for obtaining miracidia from livers of mice infected with *S. mansoni* have been described before, as has a semi-quantitative bioassay for miraxone[1]. In this assay, miracidial responses are observed stereomicroscopically and scored 1 to 4+ after wells (1.5 cm diameter, each containing 50–100 miracidia in 0 3 ml of commercial spring water) are "point-inoculated" at the edge with 20 μl of test fluid; 4+ denotes strong, persistent miracidial aggregation and excitation around the zone of inoculation, whereas lower scores denote progressively less pronounced aggregations, as previously detailed[1]. Replicate experiments yielded similar results.

We have used the bioassay to detect miraxonal activity at each step of a procedure designed to isolate and purify miraxone(s) from "snail-conditioned water" (SCW) prepared by confining 100 snails (12–16 mm diameter) in 100 ml of food-free distilled water for 5 h at 24±1 °C. SCW was filtered through paper and successive batches were pooled and stored at −10 °C until processed. Lyophilising SCW yielded "snail-water powder" (SWP) which was extracted twice with chloroform–methanol, 2 : 1 (125 ml per g SWP) The pooled extract was shaken with distilled water[8], and the resulting lipid-free upper phase—containing most of the miraxone originally present in SWP—was separated, dried, dissolved in distilled water, and then passed through a Diaflo UM-05 ultrafiltration membrane (Amicon) retaining material of molecular weight >500. The filtrate was fractionated in Sephadex G-10 gel using distilled water as eluant, and a single band of miraxone was detected at V_e/V_0 (elution volume/void volume)=1.3–2.0. This miraxone was further purified by ascending paper chromatography in two successive solvent systems. (1) butanol–methanol–acetate–water, 4 : 8 : 1 : 1 (R_f, or migration relative to solvent front, of miraxone=0.5–0.8); and (2) isopropanol–pyridine–water, 7 : 7 : 2 (R_f of miraxone=0.3–0.55) A sample of 5 l of SCW yielded about 1 g SWP from which we obtained about 26 mg miraxone after the full purification process.

When eluted from the second paper chromatogram, the miraxone was soluble in water and alcohol; insoluble in ether, hexane and chloroform; heat stable (120 °C for 4 h); stable at all *p*H levels; strongly deliquescent; odourless, and non-volatile It formed a glassy film when dried. The product apparently lacked amino groups as it proved negative by ninhydrin spot tests and by fluorescamine analysis[9]. Tests for reducing sugars and fatty acids were negative. Mass spectroscopy to determine molecular weight failed because the material was non-volatile.

The purified miraxone contained 8.9–21.6% magnesium, 28.3–35.4% chlorine, 0–8.5% carbon, 5.8–6.0% hydrogen and 0 5–0.6% nitrogen (% by weight; range of two separately purified samples). Control experiments revealed that (1) distilled water, processed through the purification scheme described above, yielded no miracidial stimulant and only 0.03% of the magnesium present in SCW-purified miraxone, and (2) the carbon and nitrogen in miraxone probably represented contaminants from chromatography.

A grey residue, remaining after purified miraxone was ashed, contained 28% magnesium by weight but only traces of other metals When this ash was dissolved in 0.5 N HCl, dried, and dissolved in water, its miraxonal activity (as measured at serial twofold dilutions[1]) resembled that of the miraxone before ashing. By contrast, when SWP was ashed and dissolved as described, its activity decreased, perhaps

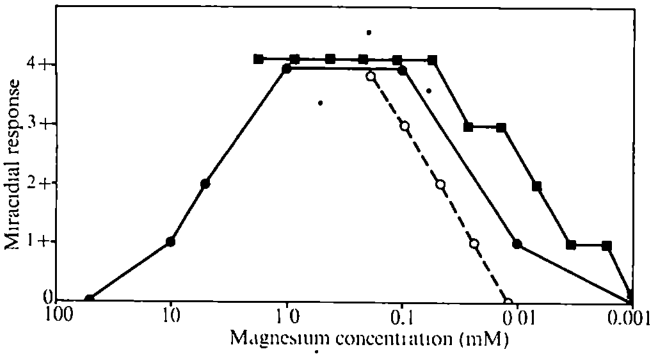

Fig. 1 Responses (1 to 4+) of *S. mansoni* miracidia, plotted against magnesium concentrations in serial dilutions of: commercial magnesium chloride (●); a typical sample of *B. glabrata*-conditioned water (○), and miraxone purified from *B. glabrata*-conditioned water as described (■). Dilutions were all made with distilled water. Magnesium concentrations were determined by atomic absorption spectrophotometry The decreased miracidial response at concentrations of commercial magnesium chloride above 1 mM represents an unexplained effect also observed with some highly concentrated preparations of miraxone. Tests with magnesium sulphate yielded curves resembling that shown for magnesium chloride.

because ashing may alter the ratio of magnesium to interfering cations (see below).

The purified miraxone migrated 9–12 cm cathodally in high voltage paper electrophoresis (30 min at 3 kV; buffer, pyridine-acetate-water, 400 : 16 : 3584; *p*H 6.42; Whatman 3MM paper); therefore, the miraxone is a cation at this *p*H. Electrophoresis removed chloride from the miraxone but left the biological activity unchanged.

The above findings suggested that the material purified from SCW is magnesium chloride, and that the biologically active miraxone is magnesium ion. This hypothesis is strengthened by three other lines of evidence First, aqueous solutions of $MgCl_2$ or $MgSO_4$ (1.0–0.1 mM) evoked strong miracidial responses (Fig. 1), while solutions of $CaCl_2$, KCl, K_2SO_4 and NaCl (1.0–0.001 mM) elicited no miracidial response. Second, when we processed magnesium chloride ($MgCl_2.6H_2O$, ACS grade, Fisher Scientific) through the purification scheme (starting with chloroform–methanol extraction), miraxonal activity purified exactly as the SCW-derived miraxone at each step. Finally, $MgCl_2.6H_2O$ contains, by calculated weight, 12% magnesium, 35% chlorine and 5.9% hydrogen—values close to those of the purified miraxone.

Several additional findings favour magnesium ion as the major miraxone in SCW. Fresh SCW, which induces 4+ miracidial responses, contained an average of 0.24 mM magnesium (range: 0.17–0.35 mM). More important, the dilution–response curves for SCW and for purified miraxone parallel that of magnesium chloride at comparable magnesium concentrations (Fig. 1). The magnesium content of SCW, therefore, can account for the miraxonal activity of this solution assuming that all or most of the magnesium is chemically 'available' as a miracidial stimulant and that other components of SCW do not interfere with miracidial stimulation by magnesium.

To test for other miraxones in SCW, the magnesium concentration in test wells containing miracidia was made up to 0 33 mM by adding $MgCl_2.6H_2O$ 5 min before challenging the miracidia with SCW of known activity. Although the miracidia swam normally, they did not respond to the SCW. This result is consistent with magnesium being the only major miraxone in SCW. The increased background magnesium (now greater than that of SCW) would have eliminated any magnesium gradient from the inoculated SCW, but this should not have affected gradients of other miraxones if any such were present. We cannot, however, deny that magnesium could nonspecifically inhibit miracidial responses to other hypothetical miraxones.

The miraxonal activity of a solution may depend in part on the concentrations of inorganic ions other than magnesium. For example, the activity of SCW[1] and of a 0.47 mM magnesium chloride solution is reduced if 34 mM NaCl is added to these solutions before bioassay. Although we do not understand the basis for this effect, the blockage suggests that direct comparison of the miraxonal activity of purified and crude materials may be misleading because interfering ions or other substances may be present in the crude preparations.

Although we do not exclude the possibility that organic molecules in crude SCW contribute to some of its miraxonal activity, our findings show that magnesium ion accounts for most of that activity. Since previous evidence indicates some nonspecificity between the snail species releasing miraxone(s) and the species of miracidium responding[1–3], perhaps the miracidia of many species of trematode respond to magnesium. This response may be a requisite part of the miracidial host-finding process. It remains to be shown how snails release magnesium, how miracidial behaviour is altered by magnesium and how this information can be used to interrupt the transmission of schistosomiasis.

We acknowledge preliminary research by Dr Robert Murphy, and the technical assistance of Ms Christine A. Burns and Ms Nora S Anderson. This study was supported in part by a research grant, and by a research career award to E C, from the US Public Health Service.

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Received December 31, 1975: accepted February 24, 1976.

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Temperature dependence of biochemical oscillations in cell suspensions of *Dictyostelium discoideum*

ON a substratum the aggregation of amoebae of *Dictyostelium discoideum* often occurs in pulses because the aggregation centres release the chemotactic signal periodically[1,2]. The signal is believed to be cyclic AMP[3,4]. Cyclic AMP oscillations have been observed in suspensions of aggregation competent cells of *D. discoideum*[5]. Since circadian rhythms are largely compensated for temperature effects[6] the temperature dependence of other biological rhythms is of general interest.

In suspensions of *D. discoideum* cells, changes in absorbance accompanying periodic cyclic AMP production can be recorded[5,7]. These changes occur as an initial series of spikes followed by sinusoidal oscillations[7]. We have studied the sinusoidal oscillations since they were found to

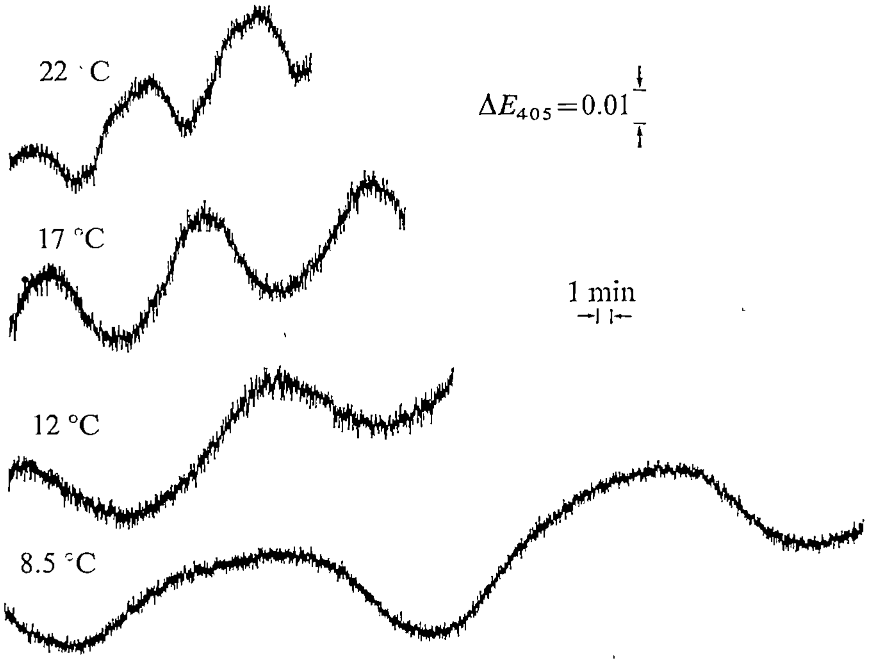


Fig. 1 Oscillations in cell suspensions of *D. discoideum* at different temperatures. Amoebae of the axenic strain Ax-2 (clone 214) were cultivated at 23 °C on nutrient medium supplemented with 1.8% maltose[11] and collected at cell densities of 0.2 to 1×10^7 ml^{-1}. The cells were washed three times in the cold with 0.017 M Soerensen phosphate buffer, *p*H 6.0, adjusted to 1×10^7 cells ml^{-1} in buffer and shaken for 4 h at 23 °C. After this the amoebae were centrifuged, adjusted to 5×10^7 cells ml^{-1} in buffer, transferred into a cuvette with an optical path of 1 cm, and agitated by bubbling oxygen at a flow rate of 24 ml min^{-1} through the suspension. With a Zeiss PM6 spectrophotometer the optical density at 405 nm was recorded. Changes in absorbance seem to be due to changes in light scattering[7] and indicate changes in shape or size of cells. The absorbance decreases towards the top. The starting temperature was 22 °C and after sinusoidal oscillations began (after ~ 1 – 2 h) the temperature was either kept at 22 °C or slowly shifted to 17 °C, 12 °C, or 8.5 °C. In all experiments the actual temperature in the cuvette was determined

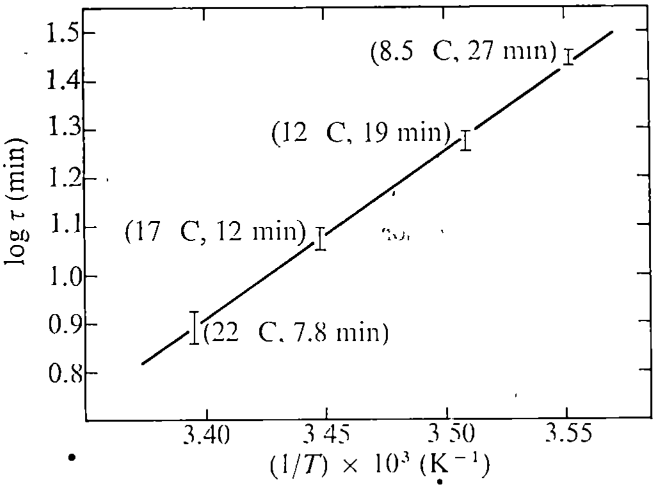
proceed for more periods than the spikes. Sinusoidal oscillations of amoebae of *D. discoideum* strain Ax-2 (clone 214) have been observed in the temperature range from 7 °C to 24 °C. As shown in Fig. 1, by decreasing the temperature from 22 °C to 8.5 °C the oscillation period increases from 7.8 to 27 min. The dependence of the oscillation period τ on the temperature T can be described by the Arrhenius equation (Fig. 2), and an activation energy for the oscillating system of $E=16$ kcalorie is obtained.

Temperature does not affect the form of sinusoidal oscillations (Fig. 1). At any temperature the part of the wave from the maximum to the minimum in absorbance is longer than that from the minimum to the maximum. The amplitude does not decrease with decreasing temperature, on the contrary, in some experiments higher amplitudes were obtained at lower temperature.

Fig. 2 Dependence of the oscillation period on the temperature. The Arrhenius equation was applied in the form

$$\log \tau = (E/2.3RT)+\text{constant}$$

(where τ is the oscillation period, R the gas constant, and T the absolute temperature) and $\log \tau$ was plotted against T^{-1}. The Celsius temperature and the period of oscillation are given in parentheses. At 22 °C sinusoidal oscillations of Ax-2 amoebae in suspension ceased after about 20 periods, and the period length decreased from the beginning to the end by 10 to 20%. At each temperature the period length was determined between 5 and 10 oscillation periods from the beginning. The data represent the range of period lengths evaluated from at least 4 oscillation periods at each temperature



During the course of these studies we learned from Nanjundiah that he and his colleagues[8] have investigated the temperature dependence of the frequency of periodic movement steps during the aggregation of amoebae of *D. discoideum* strain NC-4H on agar. Although at any temperature the oscillation period of NC-4H amoebae on agar is shorter than that of Ax-2 amoebae in suspension, the activation energy is the same under both conditions: 15.8 kcalorie (ref. 8) compared with 16 kcalorie. This result is in accord with the assumption that the same biochemical oscillator underlies rhythmic signalling of aggregation centres on agar as well as periodic activities in cell suspensions.

Nanjundiah *et al.*[8] interpreted E as the activation energy of a rate-limiting step in the biochemical oscillation. Alternatively a linear Arrhenius plot for a network of reactions results if the activation energies of all individual steps are similar. The activation energy of 16 kcalorie is in the range of those calculated for enzyme-catalysed reactions[9]. A nonenzymatic chemical oscillator, the Belousov–Zhabotinskii system, shows an even stronger temperature dependence of the oscillation period[10], corresponding to an activation energy of 26 kcalorie (as calculated from the data of ref. 10).

I thank Drs G. Gerisch, F. Grun and V. Nanjundiah for stimulating discussions. This work was supported by the Schweizerische Nationalfonds.

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Received February 4, accepted February 20, 1976

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Effect of temperature on morphogenetic oscillations in *Dictyostelium discoideum*

WHEN their food supply is exhausted, single amoebae of the cellular slime mould *Dictyostelium discoideum* aggregate[1]. On a two-dimensional substrate such as agar, this aggregation is the result of a succession of periodic coordinated movement steps directed towards a group of amoebae—the "centre", which may in fact lie anywhere in the field of cells Recent theoretical[2,3] and experimental[4-7] work suggests that this process can be explained as follows (1) The centre produces and releases cyclic AMP (the signal) in an oscillatory manner, and (2) other cells in the field respond chemotactically by moving up cyclic AMP gradients and relay the signal progressively outwards. By means of time-lapse cinematography we have looked at the oscillation frequency ν during the aggregation of small drops (about 1,000 cells in a drop of diameter 1 mm) of *D. discoideum* cells (strain NC-4H) aggregating on hydrophobic agar[8] at various temperatures, and have found that the oscillation frequency increases with temperature (Fig. 1).

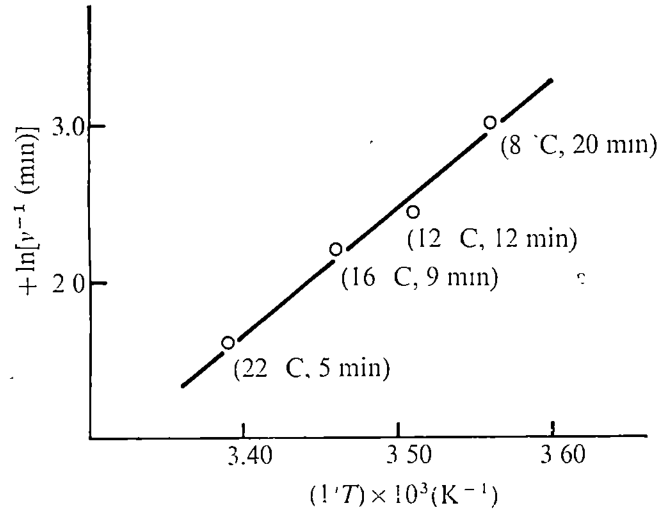

Fig. 1 T is the absolute temperature and ν the frequency of oscillation. Experimental points are fitted to the curve $\ln(\nu^{-1}) = \ln(\nu_0^{-1}) + (\Delta E/R)T^{-1}$, with R equal to the gas constant. Figures in parentheses indicate the temperature (°C) and period of oscillation. The amoebae were deposited in a drop of Bonner's salt solution[11] on agar, and the agar plate was kept in the temperature-controlled room for 2–3 h before aggregation began. Temperature varied within ±0.5 °C of the recorded means. Periods were recorded as differences in times of initiation of wave propagation at the centre, each such initiation having the appearance of a quick contraction. Mean frequencies were estimated from two experiments at each temperature with about 15 oscillations recorded during each aggregation A sample run of periods, recorded at 12 °C, reads 13, 12, 12, 13, 13, 12, 12, 12, 11, 12 min. The aggregates were concentric, that is, morphologically similar in all experiments

These amoebae aggregate and form viable spores (as tested at 22 °C) down to 8 °C. The Arrhenius plot ($\ln\nu^{-1}$ against $\ln T^{-1}$) is linear, corresponding to a frequency factor $\nu_0 = 2\times10^9\ \mathrm{s}^{-1}$ and an activation energy $\Delta E = 15.8$ kcalorie mol^{-1}. ΔE can be interpreted as the activation energy of a (hypothetical) rate-limiting step in the overall scheme for the biochemical oscillation. It is noteworthy that this value of ΔE is in the range of activation energies for non-transport enzyme-catalysed reactions[9], whereas those for known specific transport processes are much smaller[10]. In parallel with the increase in oscillation frequency, there is also an increase in the mean speed of amoeboid movement (not shown) Thus, other factors being the same, the number of pulses needed for a field of cells to aggregate ought to be only weakly temperature dependent. In fact, if we consider just those cases (four in all) where oscillations could still be observed till late in aggregation, this number was 20 at 12 °C, 19 at 16 °C and 17 at 22 °C. Rigorous substantiation of this last result would suggest that there is an underlying oscillator controlling movement, possibly coupled to the one involved in signal generation.

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Received December 4, 1975, accepted February 20, 1976.

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External cyclic AMP-dependent protein kinase activity in rat C-6 glioma cells

IN certain eukaryotic cells β-agonistic catecholamines bind to membrane-associated hormone receptors, and stimulate the intracellular synthesis of cyclic AMP by activation of the adenylate cyclase[1] In C-6 rat glioma cells stimulated with noradrenaline this increase of cyclic AMP leads to, or is correlated with, a morphological change in the cells[2]. When the intracellular cyclic AMP concentration has reached a maximum, it returns to basal values[2] due to an energy-dependent excretion of cyclic AMP from the cell into the external medium[3,4], similar to that found in several mammalian systems[5-7]. It is possible that the excreted cyclic AMP interacts either with an external, glial-membrane bound cyclic AMP receptor or with the surface of the surrounding cells, for example neurones. We report here that intact, cultured glial cells seem to have a cyclic AMP-dependent protein kinase on their external surface which transfers γ-phosphate of ATP to an external acceptor protein (histone). This protein kinase activity is stimulated indirectly by the addition of noradrenaline to the cells The physiological significance of this process is unknown

We first tested the effect of extracellular cyclic AMP on the ability of a protein kinase activity of intact C-6 cells to phosphorylate an exogenous substrate. Figure 1 shows the phosphorylation by C-6 cells of added external histones as a response to incubation in the presence of 5×10^{-6} M cyclic AMP. These data suggest that actively growing glial cells have a cyclic AMP-dependent protein kinase, probably on the external cell surface, which can be stimulated from the outside of the membrane The same experiment showed that a small fraction of ^{32}P radioactivity remained with the cells and that there was no significant difference between stimulated and unstimulated cells with respect to this accumulation (data not shown).

There are two possible objections to the experiment

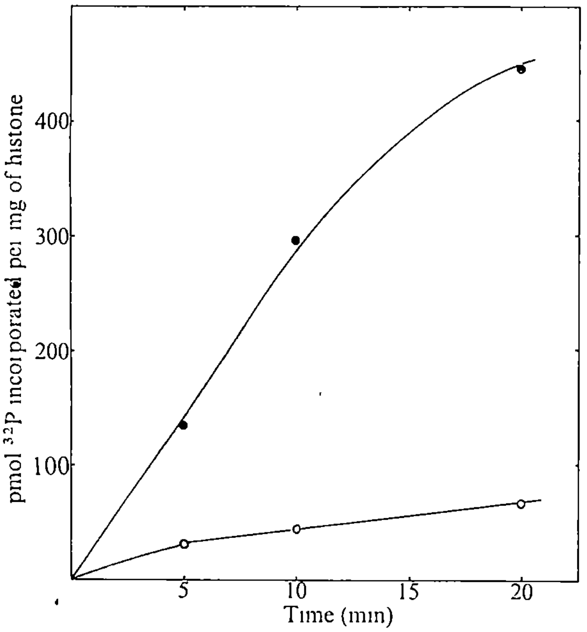


Fig. 1 Activation of external protein kinase-mediated incorporation of ^{32}P into histone. C-6 rat glioma cells (flow laboratories), originally derived by Benda *et al.*[9], were grown routinely in 60-mm Petri dishes with Ham's F-10 medium supplemented with 15% horse serum, 2.5% foetal bovine serum and gentamicin (50 μg ml^{-1}) in an atmosphere of 90% air and 10% CO$_2$ at 37° C. All experiments were performed with confluent cultures (about $3-4\times10^6$ cells per plate) since it has been shown that cell–cell contact is required for maximal increase of internal cyclic AMP after hormone treatment[10]. To measure the external protein kinase activity, the cells were first washed three times with prewarmed Dulbecco's phosphate-buffered saline (PBS), then 1 ml of reaction buffer (50 mM MOPS buffer, *p*H 7.2, 5 mM MgCl$_2$, 0.125 mM CaCl$_2$, 125 mM sucrose, 7 mM mercaptoethanol) was added to start the incubation. The reaction buffer included γ-^{32}P-ATP (specific activity 1.3×10^3 c.p.m. pmol^{-1}), prepared according to Glynn and Chappel[11], and calf thymus histones (300 μg ml^{-1}, Calbiochem). If the effect of cyclic AMP was to be measured, the reaction buffer also contained 5×10^{-6} M cyclic AMP. During the experiment the dishes were placed on a constantly moving, shaking table. After the periods indicated at 35° C, the buffer was removed and the cells were washed with 1 ml of cold PBS. Buffer and washings were combined and centrifuged (2 min, 1,500*g*), and protein in the supernatant was precipitated with 10% trichloroacetic acid (TCA). The precipitates were collected on Whatman GF/A glass fibre filters. The filters were washed six times with 5 ml of 5% TCA and once with 3 ml of methanol, dried and counted in a liquid scintillation counter. The cells were washed three times with cold PBS and lysed with 1 ml of 0.1% sodium dodecyl sulphate. The cell lysates (1 ml) were counted in 10 ml of a toluol–Triton X-100 mixture (9 parts toluol+1 part Triton X-100 containing 0.5% PPO). Each point represents the mean of three determinations: ●, 5×10^{-6} M cyclic AMP; ○, without cyclic AMP.

shown in Fig. 1. Perhaps the cyclic AMP added to the culture medium penetrates into the cells and activates an internal protein kinase. This is unlikely because it seems improbable that cyclic AMP enters glioma cells—it does not induce the characteristic morphological alterations which are a prompt response of these cells to the addition of dibutyryl cyclic AMP or catecholamines (our unpublished observations). It is also possible that a small fraction of the cells disintegrate during incubation. Cytoplasmic cyclic AMP-dependent protein kinase could then phosphorylate the added histones. This possibility is also unlikely because microscopy has revealed no gross alteration of cellular integrity. Contamination is ruled out by the experiments summarised in Table 1. These controls show that less than 2% cyclic AMP-dependent protein kinase activity was found in the incubation buffer after contact with the cells. The time course shown in Fig. 1 also excludes the possibility of contamination. The external protein kinase is most active at cyclic AMP concentrations higher than 10^{-8} M and has a *p*H optimum of about 7.2 (data not shown).

Table 1 Control experiments

	pmol ^{32}P incorporated per mg of histone per 10 min	
	Reaction buffer without cyclic AMP	Reaction buffer with 5×10^{-6} M cyclic AMP
Cell-free supernatant	5.05±0.85	5.25±0.44
Standard reaction procedure (with cells)	38.42±2.24	302.56±4.37

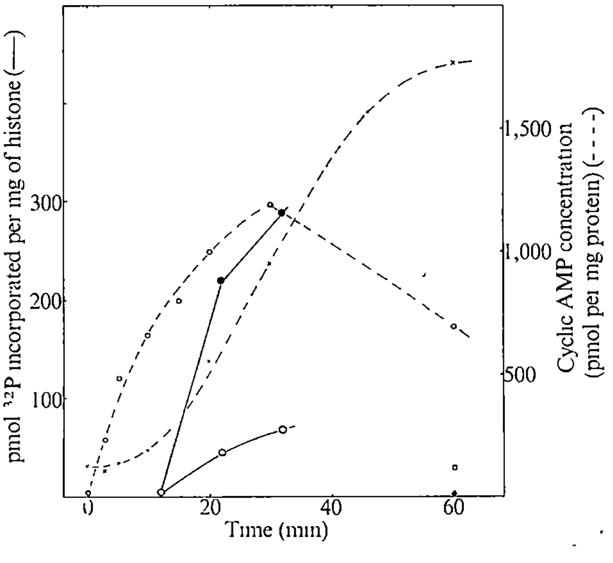
Each value represents the mean ±s.e. of five determinations.

To show that the external protein kinase activity is really located at the cell surface we added the ^{32}P acceptor protein to the reaction buffer after having incubated the buffer with growing cells. Incubation buffer without histones was removed from the cells after 10 min of incubation at 35° C in the conditions described for Fig. 1. The sample was centrifuged for 5 min at 1,500*g* and protein kinase activity in the cell-free supernatant was determined after addition of histones. The radioactivity in TCA-precipitated histones was determined after 10 min of incubation as described for Fig. 1 (cell-free supernatant). These values are compared with values obtained when histones were present with the cells during the first 10-min incubation and the second incubation not performed (standard reaction procedure).

Secretion of cyclic AMP from C-6 rat glioma cells into the medium was originally described as a response to treatment with L-isoproterenol[3,4]. We show here that noradrenaline also causes secretion of cyclic AMP from glioma cells.

Figure 2 shows the intracellular and extracellular levels

Fig. 2 Activation of external protein kinase by noradrenaline. Noradrenaline (10^{-4} M, Sigma) was added to confluent C-6 glioma cells growing in 60-mm dishes with 1 ml of medium. At the times indicated, cyclic AMP was extracted with 5% TCA as described by Oey[2] and cyclic AMP concentration was determined according to Gilman[13]. For protein determination[12], bovine serum albumin (Sigma) was used as standard. To measure the ability to transfer ^{32}P into histone in the presence of noradrenaline the cells were preincubated in Ham's F-10 medium for 12 min. Then the medium was replaced with incubation buffer containing 10^{-4} M noradrenaline and the protein kinase activity was measured as described for Fig. 1. Each value represents the mean of duplicate determinations on two cell populations. —●—, Protein kinase activity in the presence of noradrenaline; —○—, in the absence of noradrenaline; - - ○ - -, internal cyclic AMP concentration in the presence of noradrenaline, ×, external cyclic AMP concentration with noradrenaline; △, internal cyclic AMP concentration without noradrenaline; □, external cyclic AMP concentration without noradrenaline. The cyclic AMP efflux from growing C-6 cells into incubation buffer occurs at nearly the same rate as into medium (data not shown).



of cyclic AMP after incubation in the presence of 10^{-4} M noradrenaline. After a short lag period, cyclic AMP appeared in the external medium The external protein kinase could also be activated by treatment of the cells with noradrenaline. This is most clearly demonstrated by the experiments shown in Fig 2.

The best known and probably the main receptor for cyclic AMP in eukaryotic cells is the regulatory subunit of a protein kinase After binding cyclic AMP, the regulatory subunit is released from the catalytic subunit, thereby stimulating the activity of the enzyme[8].

We thank Professor R. Knippers and Drs T Lindl and A Powling for helpful discussions. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

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H–2 region product as determinant in immune cytolysis of syngeneic tumour cells by anti-MSV T lymphocytes

THE existence of cytolytic T lymphocytes (CTL) specific for antigenically related tumour cells during oncornavirus-induced oncogenesis in the primary host is well documented. They exist in murine sarcoma virus (MSV) tumour-bearing mice, during and after the tumour rejection[1], and in Friend virus-infected mice, notably in the spleen of resistant C57BL/6 (ref. 2) It is probable that they play some role in tumour cell destruction *in vivo*[3] and it can be supposed that the antigens of the tumour cell surface recognised by CTL are at least, for the most part, viral proteins or glycoproteins. It was suggested that CTL reacting with virus producer cells in non-oncogenic systems[4], or with hapten-modified cells[5,6] recognise an H–2-modified antigen. Therefore, it was of interest to determine whether the same situation exists in an oncornavirus tumour system. It is already known that anti-MSV CTL are much more efficient against syngeneic than against allogeneic tumour cells[7,8], indicating that H–2 antigens may play some role in the CTL–target cell interaction. We report here that the H–2 specificities of the tumour cell surface determine the interaction with syngeneic anti-MSV CTL, the reacting antigen being either an H–2 specificity modified by the virus, or, less probably, a virus-induced antigen modified by an *H–2* region product.

Anti-MSV CTL derived from different inbred lines and their F_1 hybrids or congenic partners were tested against tumour cells of various H–2 specificities. CTL were taken from the spleen 10–16 d after virus inoculation in BALB/c (*H–2*^{d}), B10.D2 (*H–2*^{d}), BALB B (*H–2*^{b}), C57BL/6 (*H–2*^{b}) and (BALB/c×C57BL/6)F_1 (*H–2*$^{b/d}$). All mice were from our colony except BALB.B (Dr F. Lilly, Albert Enstein College, Bronx, New York) BALB/c (C) and BALB.B differ only at the *H–2* region of group linkage IX (chromosome 17). A similar situation exists between C57BL/6 (B6) and B10.D2, the latter being congenic to C57BL/10, closely related to B6. Tumour cells were YC8 and LSTRA in C, and MBL2 and FBL3 in B6 YC8, LSTRA and MBL2 were Moloney virus-induced lymphomas; whereas FBL3 was induced by Friend virus. All of them are FMRGi(+) cells as defined serologically. MBL2 and FBL3 reacted similarly with B6 anti-MSV CTL as do LSTRA and YC8 with C anti-MSV CTL. The CTL activity was tested using the chromium release test (CRT) carried out at 100 : 1 CTL–target cells ratio with an incubation period of 6–16 h as described previously[1]. It was demonstrated earlier that in the MSV system the cytolytic activity is entirely due to T cells[1,9]. Results (Table 1) show that B6 anti-MSV CTL are strongly cytolytic for B6 and only weakly for C targets, the reverse situation being observed with C anti-MSV CTL. (B6×C)F_1 CTL which possess both *H–2*^{d} and *H–2*^{b} determinants of their parental strains are fully effective against *H–2*^{d} and *H–2*^{b} targets CTL obtained from congenic lines behave exactly like their H–2 identical partner: BALB.B CTL lyse B6 better than C tumour cells, whereas B10/D2 are mainly effective against C cells The use of congenic lines is particularly demonstrative of the H–2 participation in CTL-induced cytolysis since they differ from their congenic partners only by the *H–2* region. In experiment 1 a weak CTL activity exists for allogeneic targets, also found occasionally in other unpublished experiments. An H–2 incompatibility does not therefore completely abolish the CTL reaction, but as a rule the allogeneic reaction remains very weak as shown in quantitative experiments (F. Plata *et al*, unpublished) One can therefore conclude that H–2 specificities are determinant in the reaction, H–2 compatibility between CTL and the target being necessary for a fully efficient immune cytolysis. Two hypotheses can be proposed to explain this phenomenon. (1) CTL recognise MSV antigens by a specific determinant, but they need at the same time an identical H–2 on their surface and on the target cell to become fully cytolytic. In other words two different structures are involved separately on the tumour cell: H–2 antigens and the MSV specificities, (2) anti-MSV CTL recognise on the tumour cell surface only one structure which is either an H–2 antigen modified by a viral product, or a viral antigen modified by an H–2 region product. According to the first hypothesis, CTL from F_1 hybrids bearing the H–2 specificities of their two parental lines, F_1 immune CTL must react with any parental cells possessing the MSV-induced antigens. On the contrary, if the second hypothesis were correct, F_1 CTL would react only with target cells sharing an identical H–2 with the immunising parental tumour cells.

Table 1 Specific chromium release (%) by MBL2 (B6) and LSTRA (C) tumour cells attacked by anti-MSV CTL from different strains

Anti-MSV spleen cells from:	Target cells MBL2 Expt 1	MBL2 Expt 2	LSTRA Expt 1	LSTRA Expt 2
BALB/c (C)	7	0	17	34
C57BL/6 (B6)	34	26	7	0
(C×B6) F_1	39	—	16	—
BALB B	45	—	8	—
B10.D2	7	—	24	—

(C×B6)F_1 were therefore immunised either by MSV inoculation (which induces F_1 transformed cells *in vivo*) or by 10^5 MBL 2 or FBL 3(B6) or LSTRA (C) tumour cells given subcutaneously. CTL were taken from the spleen about 2 weeks later and tested using the CRT against B6 and C targets Results show (Table 2) that hybrids immunised against the virus produce CTL reacting with both MBL2 and YC8. On the contrary, hybrids immunised against C or B6 tumour cells produce CTL which react only

Table 2 Cytolytic activity of (C×B6)F$_1$ CTL for B6 and C tumour cells (% specific chromium release)

Immunisation by:	Target cells MBL2 (B6)	 YC8 (C)	 LSTRA (C)
MSV inoculation	38	—	16
	13	8	—
	36	21	—
LSTRA (C) tumour cells	6	—	34
	4	—	26
	0	—	12
MBL2 (B6) tumour cells	7	0	—
FBL3 (B6) tumour cells	13	0	—
	12	0	—

with the immunising cells or antigenically related cells of the same line. One can conclude that H–2 compatibility between CTL and the target is not enough to permit immune cytolysis. Hybrids (*H–2*$^{b/d}$) which received the virus and developed MSV tumour cells have been immunised with MSV antigens associated with (or modified by) H–2^{b} and H–2^{d} on the tumour cell surface. Therefore, they react with YC8 (*H–2*^{d}) as well as MBL2 (*H–2*^{d}) lymphoma cells. On the other hand, hybrids which received parental tumour cells have been immunised only against tumour antigens associated with the H–2 specificity of the immunising line, and do not therefore react with the tumour antigen of the other parental line. At this stage in the experiments it seems highly probable that anti-MSV CTL recognise only one structure on the target cell surface—an antigen which is different according to the H–2 of the host.

This structure could be an H–2-modified antigen due to the interaction of viral proteins or glycoproteins with H–2 molecules on the tumour cell surface, as proposed for non-oncogenic virus systems[4] and Hapten-modified cells[5,6]. According to this hypothesis H–2 and MSV antigens must be closely associated and a preincubation of the targets with related H–2 antibodies ought to block the activity of anti-MSV CTL. Another possibility would be that the virus-induced antigens could be modified by a product of the H–2 region, as proposed in experiments using CTL directed against non-H–2 specificities on the normal cell surface[10] In this case H–2 and MSV antigens are not necessarily associated on the tumour cell surface, and anti-H–2 antibodies would not necessarily block activity. To demonstrate a close topographical relationship between H–2 and MSV antigens, C anti-B6 and B6 anti-C antisera prepared by repeated inoculations of normal spleen cells. These sera were used either unabsorbed or after absorption *in vivo* on congenic spleen cells to provide H–2 and non-H–2 typing sera. For example, C anti-B6 absorbed on BALB.B provides a 'non-H–2 typing serum', whereas the same sera absorbed on B10/D2 provides an 'H–2 typing serum'. Target cells (2×10^{4}) were incubated for 30 min at 4 °C in 0.2 ml 1/10 diluted typing serum, then used in the CRT as above. Controls were incubated either with normal mouse serum or with unrelated anti-H–2 typing serum. Results (Table 3) show that unabsorbed and anti-H–2 typing sera block the activity of anti-MSV CTL, whereas non-H–2 typing sera are devoid of blocking activity. Furthermore, a BALB/c anti-BALB.B typing serum which reacts only with H–2 is fully blocking. Anti-Thy1–2 (AKR anti-C3H/He) incubated with the Thy1–2(+) MBL2 lymphoma cells did not inhibit their cytolysis by anti-MSV CTL. In addition, unrelated anti-H–2 (for example, B6 anti-C on B6 cells attacked by B6 anti-MSV CTL) are not blocking. It is clear therefore that anti-H–2 have a definite and specific blocking activity for anti-MSV CTL. This result indicates a close topographical relationship between H–2 and MSV antigens and favours the hypothesis of an H–2-modified specificity at the surface of oncornavirus-infected cells. One cannot definitely rule out the hypothesis, however, of a steric hindrance by anti-H–2 antibodies, due to the proximity of H–2 and MSV antigens, without structural relationship between them. Note also that repeated washings of the target cells after incubation with anti-H–2 clearly altered their blocking activity. It is therefore not possible to draw a definite conclusion from these experiments.

H–2 specificities are clearly determinants in the interaction of anti-MSV CTL and syngeneic tumour cells. Again the H–2 region seems to have a major role in an immunosurveillance phenomenon as reported previously for non-oncogenic virus infections, including lymphocytic chriomeningitis[4], vaccinia[11] and ectromelia[12], after immunisation by hapten-modified cells[5,6]; and in the immune response against non-H–2 antigens of normal lymphoid cells[10]. Similar results have also been found in tumours induced by Friend leukaemia virus[13] and during *in vitro* stimulation of anti-MSV CTL by tumour cells (F. Plata, personal communication). It is generally assumed that the antigen reacting with CTL is an H–2 modified specificity[4,6,11,12]. MSV and Friend systems are especially interesting in this case, since the viral polypeptides reacting with H–2 molecules can probably be determined. Further studies are in progress to try to clarify this point and to define the H–2 products involved· H–2D, H–2K or Ia determinants. Preliminary results show that H–2D antigens are especially important.

Another hypothesis must be considered. It has been shown that an H–2 compatibility betwen CTL and targets is necessary in the CTL reaction directed against non-H–2 antigens of normal lymphoid cells[10]. In this situation H–2 and non-H–2 determinants are presumably not on the same structure, and the hypothesis of an *H–2* region product specifically modifying non-*H–2* molecules must therefore be considered[10]. The same could be true in the MSV system: it cannot be excluded, for example, that the antigenic determinants of the major glycoprotein 69/71 of the virus could be modified by an *H–2* region-directed molecule. Therefore the nature of the relationship between H–2 and virus antigens is not clearly defined, at least in the MSV system.

Whatever the nature of the H–2/MSV interactions, the existence of an H–2 barrier to the action of anti-virus producer cell CTL merits two comments of practical value. (1) The antigens reacting with CTL in MSV tumour-bearing mice are not completely understood since FMRGi, due to the Moloney virus[3,8], and MEV.SA1 antigen, due to endogenous viruses[14], are involved. From the experiments with H–2 congenic lines we can assume that, if an endogenous virus antigen is the target of the anti-MSV CTL it must be integrated in the *H–2* region and it has followed this region during the selection of BALB.B and B10.D2 congenic lines. Another, less probable, hypothesis would be that two endogenous viruses of different antigenicity are present both in BALB/c and in B6, their expression being controlled separately by different locus of the *H–2* region. It is more likely that FMRGi or other antigens, due to the MSV or to its helper virus, are the main targets of CTL, their specificities being modified according to the H–2 of the host. Note, however, that an H–2 incompatibility strongly decreases but does not completely suppress tumour

Table 3 Blocking of anti-MSV CTL induced cytolysis by preincubation of anti-H–2 antibodies with the target cells

Antisera	Specific chromium release by MBL2 targets (%)
C normal serum	18
C anti-B6	0
C anti-B6 absorbed in BALB.B	17
C anti-B6 absorbed in B10.D2	4
C anti-BALB B	0
B6 anti-C	19
AKR anti-Thy 1–2	19

cells lysis by CTL, since highly potent CTL can be weakly efficient against allogeneic cells of different H-2 (refs 8 and 14). This could be due to the existence of different subpopulations of immune CTL, some of them reacting, for example, with antigens unrelated to H-2. (2) If HLA has the same role in humans as H-2 in mice, study of antitumour cell reaction due to CTL in cancer patients must be carried out in autologous conditions or, at least, between CTL and target cells sharing HLA determinants. Negative results would otherwise be of little significance.

Supported by contracts from INSERM and DGRST.

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Received January 28, accepted March 9, 1976.

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Plasmin-mediated agglutination by concanavalin A of 3T3 cells cocultured with SV40–3T3 transformants

REICH and colleagues have reported that a variety of embryo fibroblasts in tissue culture show fibrinolytic activity after viral transformation[1,2]. This effect is attributable to the action of a plasminogen activator, which is a serine protease[3] and is present at higher concentrations in transformed than untransformed fibroblasts[4]; this activator converts plasminogen, present in the serum, to the fibrinolytic enzyme plasmin[5,6]. Certain characteristics of transformed cells, such as altered morphology and multilayering, may be plasmin dependent[7,8]. Plasmin may also induce changes in untransformed cells; this is supported by the observation that normal fibroblasts cocultured with viral transformants developed similar morphology to that of the transformed cells[8]. Furthermore, proteolytic activity released from transformed cells induced lectin-mediated agglutination, and stimulated thymidine incorporation in non-dividing cultures of untransformed fibroblasts and epithelial cells[9]. The possibility therefore exists that untransformed cells may express in coculture certain features of transformed cells as a result of environmental plasminogen activation. In support of this we report here the plasminogen-dependent enhancement of agglutination by concanavalin A (con A) of untransformed cells in coculture with transformed cells.

Table 1 Agglutination by con A of 3T3 cells in monoculture and in coculture with SV40–3T3 cells

Serum supplement (10%)	Agglutination (monoculture)	Agglutination (coculture)	Net increase
Calf	1	8	7
Foetal calf	1	8	7
Horse	2	8	6
Pig	5	8	3
Chicken	1	6	5
Human	3	7	4
Guinea pig	3	7	4
Rabbit	1	7	6

To prevent mixing, the two cell types were initially seeded separately on microscope slides anchored down with silicone grease in square Petri dishes untreated for tissue culture. They were then incubated for 24 h in Dulbecco's modified Eagle's medium+10% foetal calf serum. The slides of subconfluent cells were then thoroughly washed in serum-free medium, and transferred to new dishes for mono- or coculture in media supplemented with the sera listed. After 24 h they were washed twice and the cells were resuspended by incubating for 1 h in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) Agglutination was then carried out in complete PBS+100 μg ml^{-1} con A in conical plastic tubes which were shaken at 100 r.p.m. for 30 min at 37 °C. Agglutination was then assessed on a 0–10 scale by 10 independent observers and an average unit value obtained.

Swiss mouse 3T3 cells and SV40–3T3 transformants were used. We found that when 3T3 cells were grown in various sera they were generally only slightly agglutinable; however, when cocultured with SV40–3T3 cells they became highly agglutinable irrespective of the type of serum used (Table 1) Since contact between the two types of cell in coculture was only through the medium, this enhancement is attributable either to the release of an SV40–3T3 cell effector substance or to the activation of a medium factor by an SV40–3T3 cell activator substance. The release of one or other of these agents into the medium was demonstrated when 3T3 cells incubated in serum-free medium conditioned by four 24-h exposures to SV40–3T3 cells showed enhanced agglutination in con A compared with those incubated in unconditioned medium (Table 2). The lower level of agglutination enhancement achieved in this case when compared with 3T3 cells cocultured for a single day in unconditioned medium (Table 1) suggests that the major part of the SV40–3T3 cell agent was cell associated. This tends to support the view that enhanced 3T3 cell agglutinability required the interaction of a cell activator substance with a component of the medium. We confirmed this by failing to enhance the agglutination of 3T3 cells in the absence of serum (Table 2). Consequently, the enhancement of 3T3 cell agglutination is attributable to the interaction of an activator of SV40–3T3 cell origin with a serum component. Plasminogen is a serum component and its activated form, plasmin, is a protease with trypsin-like specificity[10] Since trypsin is known to induce con A agglutination of 3T3 cells[11] the enhanced agglutinability of 3T3 cells exposed to SV40–3T3 cell activator may be due to the conversion of serum plasminogen to plasmin. To test this we attempted to induce con A agglutination enhancement in cocultured 3T3 cells in serum-free medium to which plasminogen was added. After 24 h of coculture 3T3 cells became agglutinable, but cells incubated without plasminogen, SV40–3T3 cells, or both, failed to

Table 2 Agglutination by con A of 3T3 cells incubated in SV40–3T3 cell-conditioned medium

Serum supplement	Agglutination in unconditioned medium	Agglutination in conditioned medium	Net increase
10% calf	1	5	4
None	1	1	0

Serum-free Dulbecco's modified Eagle's medium was conditioned by exposure to SV40–3T3 cells for four separate 24-h periods, between each of which it was stored at −20 °C while the cells were allowed to recover for 24 h in foetal calf serum-supplemented medium. Before incubating with the 3T3 cells for 24 h the conditioned medium was filtered through 0 22 μm pores to remove any cells. 10% calf serum, selected for high agglutinating activity (Table 1), was added to the conditioned and unconditioned media The subsequent incubation and agglutination procedures were as described in Table 1.

Table 3 Agglutination by con A of 3T3 cells in coculture with SV40–3T3 cells in serum-free medium containing plasminogen

Cocultured with SV40–3T3 cells	Medium supplement	Agglutination
+	Plasminogen	4
–	Plasminogen	1
+	None	1
–	None	1
+	Plasminogen	1 (no con A added)
–	10% foetal calf serum	1

Cells were grown on microscope slides as described in Table 1 After washing they were incubated for 24 h in the conditions shown; lyophilised pig blood plasminogen was added at 20 μg ml^{-1}. Since cells incubated without serum were more readily detached from the substrate than those incubated with serum, the incubation in Ca^{2+}- and Mg^{2+}-free PBS was reduced to 10 min in these groups. Agglutination was carried out as described in Table 1.

agglutinate (Table 3). Consequently, enhanced agglutination is attributable to the interaction of SV40–3T3 cell activator and plasminogen. Using the casein–Agarose overlay assay[4], we found that detectable digestion of casein was produced by SV40–3T3 cells, but not by 3T3 cells, in media supplemented with any of the sera listed in Table 1. This supports the view that the SV40–3T3 cell factor is a plasminogen activator which converts added or serum plasminogen to plasmin, and that it is present at lower activity in, or absent from, 3T3 cells.

It has been reported[12] that human uterine cervix fibroblasts, normally not agglutinable with con A, show increasing agglutination as adjacent lesions of the overlying epithelium progress from dysplasia through *in situ* carcinoma to invasive carcinoma. There may be a close parallel between these changes and the plasmin-mediated enhancement of agglutination in con A of cocultured 3T3 cells reported here Since primary cultures of malignant tissues may often have high concentrations of plasminogen activator compared with normal tissues[13], the increasing agglutination of normal fibroblasts adjacent to these neoplastic lesions may be attributable to a local conversion of serum plasminogen into plasmin by increasing concentrations of tumour cell plasminogen activator. This suggests the interesting possibility that tumours may be associated with normal cells in a "quasi-malignant" state, expressing one or more features usually considered to be characteristic of malignant transformation.

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Received January 23; accepted February 24, 1976.

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Transformation of C3H/10T1/2 CL8 mouse embryo fibroblasts by ultraviolet irradiation and a phorbol ester

Ultraviolet light is the major cause of human skin cancer, and papillomas and malignant carcinomas have been induced in the skins of rats and mice by repeated exposure to it[1–5]. Pound[6] reported that although a single short exposure of mouse skin to ultraviolet light did not induce any tumours, when the single exposure was followed by repeated applications of croton oil, many tumours were induced Thus ultraviolet light acted as an initiator in the two-stage process of skin tumour development[7,8]. X rays can induce oncogenic transformation of cells in culture[9,10], and a preliminary report has appeared on the transformation of hamster embryo cells by ultraviolet light[11].

We have developed a cloned line of C3H mouse fibroblasts, C3H/10T1/2 CL8, which is non-malignant and highly susceptible to postconfluence inhibition of cell division[12]. These cells are transformed to malignancy with polycyclic hydrocarbon carcinogens and by certain alkylating agents[13], and the response can be quantified. We have also reported[14] that when these cells were treated with a subeffective concentration of polycyclic hydrocarbons followed 5 d later by a non-transforming and non-cytotoxic concentration of tetradecanoyl phorbol acetate (TPA, 0.1 μg ml^{-1})[15], transformation occurred. Thus, initiation and promotion have been achieved with our system of cultured cells. The transformed foci so obtained are of type. III, (ref. 13) which give rise to fibrosarcomas in syngeneic mice[13].

In our experiments, 2,000 C3H/10T1/2 cells were plated in 60-mm Falcon dishes in Eagle's BME medium + 10% heat-inactivated foetal calf serum (Gibco). Twenty-four hours later the cells were irradiated once in fresh medium with an ultraviolet lamp (Dazor) with doses from 10 to 100 ergs mm^{-2}. The dose of ultraviolet irradiation was measured by an ergometer (Ultra-Violet Products) in ergs mm^{-2} s^{-1}. After irradiation the cells were given fresh medium and incubated in a humidified incubator (Forma) at 37 °C in an atmosphere of 5% carbon dioxide in air. After various times some of the cultures were given TPA (0.1 μg ml^{-1}) in the medium for the remainder of the experiment. TPA (Consolidated Midland) was dissolved in spectrograde acetone and stored in amber bottles at −20 °C. It was added to the medium so that the final concentration of acetone was 0.5%. The cytotoxicity of the ultraviolet irradiation was determined by its effect on the plating efficiency of 200 cells, and the colonies were

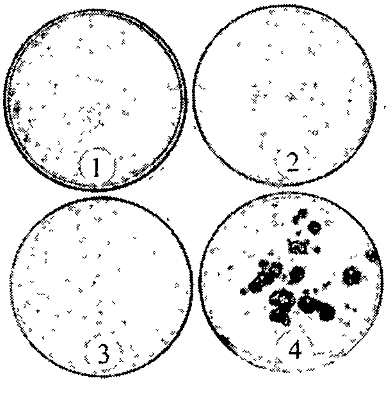

Fig. 1 Fixed and stained dishes of C3H/10T1/2 CL8 cells 6 weeks after initial treatment: 1, 0.5% acetone; 2, TPA 0.1 μg ml^{-1}; 3, ultraviolet light, 25 erg mm^{-2}; 4, ultraviolet light, 25 erg mm^{-2}, followed 48 h later by TPA 0.1 μg ml^{-1}.

Table 1 Transformation of C3H/10T1/2 CL8 cells by ultraviolet light and TPA

Treatment schedule	Plating efficiency %	No. of dishes with foci/total no. of dishes	% Dishes with foci	Transformation frequency*
0.5% Acetone	19	0/23	0	0
TPA (0.1 µg ml^{-1})	20	0/12	0	0
Ultraviolet, 10 erg mm^{-2}	19	0/12	0	0
Ultraviolet, 10 erg mm^{-2}+TPA				
48 h†	19	3/12	25	0.08
72 h	19	5/12	42	0.18
96 h	19	4/12	33	0.13
120 h	19	3/12	25	0.11
Ultraviolet, 25 erg mm^{-2}	17	0/16	0	0
Ultraviolet, 25 erg mm^{-2}+TPA				
48 h	18	10/12	83	0.83
72 h	18	11/12	92	0.69
96 h	18	10/12	83	1.0
120 h	18	9/12	75	0.35
Ultraviolet, 50 erg mm^{-2}	8	0/24	0	0
Ultraviolet, 50 erg mm^{-2}+TPA				
48 h	7	8/12	67	0.95
72 h	7	8/12	67	1.6
96 h	7	7/12	58	1.9
120 h	7	5/11	45	0.45
Ultraviolet, 100 erg mm^{-2}	1	0/18	0	0
Ultraviolet, 100 erg mm^{-2}+TPA				
48 h	1	6/15	40	3.7
72 h	1	8/17	47	2.6
96 h	1	3/12	25	1.7
120 h	1	3/11	27	1.4

The concentration of TPA used was 0.1 µg ml^{-1}.
* Percentage transformed foci per surviving cell plated
† Time after irradiation when TPA was added.

counted after 8–10 d (ref. 13). Type III transformed foci were scored 6 d after fixation with methanol and staining with Giemsa, which was 6 weeks after 2,000 cells had been plated in 60-mm dishes, as described previously[10,13].

The results are shown in Table 1. Ultraviolet irradiation at 10 and 25 erg mm^{-2} was not cytotoxic, but larger doses were No type III transformed foci were obtained in the acetone control dishes, in the dishes treated with TPA (1 µg ml^{-1}) or in the dishes treated with ultraviolet irradiation. When TPA was added to the medium 48 h or more after irradiation, however, significant numbers of transformed foci were observed (Table 1 and Fig. 1). This transformation was dose dependent for ultraviolet irradiation, with high transformation frequencies, corrected for cytotoxicity, for 100 erg mm^{-2} followed by TPA. In general, the transformation frequency decreased if TPA treatment started 96 h or more after irradiation.

These experiments show that ultraviolet light acts as an initiator of transformation in our cells, just as it did in the experiments on mouse skin[6]. This first success in achieving transformation in cell cultures with ultraviolet light will lead to important information on the mechanism of ultraviolet carcinogenesis Relevant studies, with particular emphasis on DNA repair, are in progress.

We thank Mr D. W. Brankow for technical assistance This work was supported by a contract from the National Cancer Institute C H. is an American Cancer Society Professor of Oncology.

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Received January 5, accepted February 24, 1976

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Increased procoagulant activity in cultured fibroblasts from progeria and Werner's syndromes of premature ageing

BIOLOGICAL ageing undoubtedly involves a number of complex physiological and biochemical factors but it is increasingly evident that the cultured human fibroblast will help to elucidate the fundamental nature of this process. These cells have a finite replicative lifespan that is inversely related to the age of the donor[1–3]. Moreover, physiological rather than chronological age is a critical determinant, since cultured fibroblasts from the progeria and Werner's syndromes of premature ageing have significantly reduced growth potential compared with normal, age-matched controls[3–5] Although the decreased replicative ability of cells may help to explain some of the features of progeria and Werner's syndrome, such as stunting of growth and impaired wound healing ability, it does not explain other pathological concomitants of these disorders. In particular, an explanation is needed for the premature appearance of severe atherothrombotic disease[6,7]. It has been shown that human fibroblasts contain a potent procoagulant activity called 'tissue factor' (TF)[8]. TF has the capacity to activate the extrinsic clotting mechanism through factor VII, eventually leading to thrombin generation which then promotes clot formation through fibrinogen[8,9] We report here that skin fibroblasts derived from subjects with progeria and

Werner's syndrome have markedly elevated levels of TF compared with controls.

Fibroblast strains were initiated from skin biopsies of three male subjects with classical progeria, one aged 2.5 yr and two aged 9 yr, and a 56-yr-old woman with Werner's syndrome, following informed consent of the subjects or their parents. Clinical details on two of the progeria subjects[10] and the subject with Werner's syndrome[3,6] have been published. Normal control cultures were derived from donors aged 22–76 yr, whereas abnormal controls were two subjects aged 5 yr with Lesch–Nyhan syndrome and homocystinuria. The latter two were better matched to the progeria subjects in terms of age, stunting of growth and chronic illness, all of which could possibly affect the growth of cultured cells and the amount of TF activity All cultures were initiated and propagated in our laboratory using Eagle's medium supplemented with non-essential amino acids and 15% foetal calf serum as described previously[2,10]. Subcultivation was carried out at a 1·8 split ratio counting three mean population doublings each time, until exhaustion of the replicative potential[1–3]. By this criterion, control cells had replicative lifespans ranging from 48–75 doublings, progeria cells 21–40 doublings, and Werner's strain only 15 doublings. Control cells were assayed for TF both at early and late passage, the latter 3–9 doublings before termination. Progeria cells were assayed with 9–30 doublings remaining and Werner's cells within 3–6 doublings of termination. In preparation for assay of TF activity, post-confluent sheets of cells were collected by a routine trypsinisation method[1–3] that was strictly standardised. Cells were incubated with a 0.125 trypsin solution (Difco 1:250) at 37 °C for exactly 10 min, after which the trypsin was neutralised with a 20-volume excess of growth medium containing 15% foetal calf serum. When soybean trypsin inhibitor was used to neutralise trypsin, essentially identical results were obtained in the TF assay.

Figure 1 is a combined plot of TF activities in the various fibroblast strains. TF in control cells ranged from 2 to 12 U per 10^6 cells with a mean of 5.06 U ± 3.84 (s.d.). No effect of donor age was evident. In striking contrast, progeria and Werner's cells showed significantly elevated TF activities, ranging from 23 U to a maximum of 137 U Since the anatomic site of biopsy can influence various parameters in cultured fibroblasts[3], assays on cell strains derived from skin of the anterior forearm of the 2.5- and 9-yr-old progeria subjects ($n = 16$) were compared with controls, all derived from the same anatomic site ($n = 41$). The differences remained highly significant ($P < 0.01$). Slow growth *per se* of the prematurely ageing cells could not account for these findings; when control cells were assayed during their senescent phase, with 3–9 mean population doublings remaining until termination of growth, the mean TF activity was 7.96 ± 6.82 U, still significantly different from progeria strains ($P < 0.01$). Additionally, control strains from aged adults, with growth properties similar to progeria cells[10] had substantially lower TF activities (Fig. 1). The apparent increment in TF activity during the lifespan of control cells was not statistically significant.

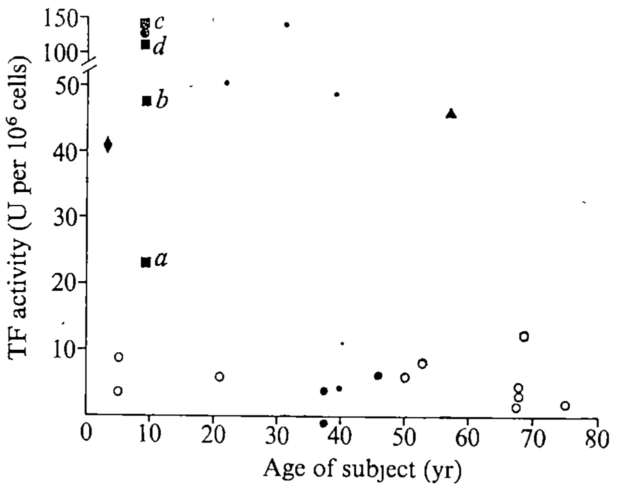

Fig. 1 TF activity of early passage fibroblasts as a function of the age of donor. The assay was slightly modified from the one-stage thromboplastin method of Zacharski and McIntyre[8]. Collected cells were suspended and rinsed twice in PBS and then in Tyrode's albumin buffer[11]. The final suspension of washed fibroblasts was adjusted to 2×10^6 cells ml^{-1}. Platelet-poor citrated human plasma obtained from healthy donors and the plasmas of patients with hereditary factor VII and factor VIII deficiency (Dade Division, American Hospital Supply Corporation, Miami, Florida 33152) were used as substrates. To carry out the clotting assay, 0.1-ml aliquots of plasma were incubated in a water bath at 37 °C, followed by addition of 0.1 ml fibroblast suspension and 0.1 ml 0.025 M $CaCl_2$. The clotting times were recorded and the procoagulant activity of fibroblasts was compared with that of rabbit brain thromboplastin (Thromboplastin reagent, Dade). We accepted arbitrarily that 1 ml of this reagent contained 1,000 U TF. Thromboplastin was serially diluted up to 1:5,120 in Tyrode's albumin buffer and the effect of each dilution was tested on the clotting time. The clotting time of normal human plasma without rabbit brain thromboplastin was approximately 210 s, whereas the sample with concentrated thromboplastin clotted in 14–15 s. Since dilution curves obtained using rabbit brain thromboplastin and fibroblasts were of a similar character, TF activity of fibroblasts was expressed in arbitrary units calculated by intrapolation. Each fibroblast suspension was assayed at a minimum of four cell concentrations. Progeria (solid symbols), Werner's syndrome ▲, and normal controls (open symbols). *a*–*d*, Single subject with progeria using individual cultures initiated from skin of various anatomic sites: *a*, anterior forearm; *b*, anterior thigh; *c* and *d*, bilateral calves. Strain ● originated from skin over the lumbar area, whereas all other cultures originated from skin of the anterior forearm. Values are means of 3–14 experiments except for *b*, *c* and *d*, which represent single observations.

Disruption of cells by sonication produced about a fivefold increase in TF activity in all strains but the differences between controls and prematurely ageing cells were maintained. This suggests that the higher levels of TF in progeria and Werner's cells relate primarily to increased content rather than increased availability. The supernatant obtained following 300*g* centrifugation of cells collected by trypsinisation and neutralised with soybean trypsin inhibitor was also assayed, but no TF activity could be detected. In contrast, control supernatants, obtained after incubating monolayers with enzyme-free buffer, contained about 10% of the total TF in the system (cells plus supernatant). These results

Table 1 Effect of cultured skin fibroblasts on clotting time of recalcified plasma in presence of normal and factor-deficient plasma

Additive	Clotting time (s) Type of plasma Normal		Factor VII-deficient		Factor VIII-deficient	
	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
Control (Tyrode's fluid alone)	186	166	227	154	527	510
Normal cells	49	80	176	145	58	61
Progeria cells	31	53	120	123	36	31

Early passage cells from 22-yr-old normal male and 9-yr-old boy with progeria were used.

are in conflict with a report using more purified trypsin[12] and suggest that different experimental techniques may be responsible. For example, the relatively crude pancreatic trypsin used in our studies may contain other enzymes such as lipases capable of digesting an essential lipid component of TF[12,13].

To determine whether fibroblast TF was specific for the extrinsic clotting mechanism (that is, activation via factor VII), experiments were carried out with intact cells using plasma from patients with hereditary deficiencies of clotting factors (Table 1). With factor VII-deficient plasma, both normal and progeric cells showed a markedly prolonged clotting time, much like the control fluid without cells. When factor VIII-deficient plasma was used, however, the clotting time was again shortened to resemble values observed with normal plasma. In total, these data strongly suggest that the extrinsic rather than the intrinsic clotting mechanism plays an important role in the TF-mediated clotting.

To determine whether TF itself was responsible for the increased procoagulant activity, cells were preincubated with specific immune globulins directed against purified TF followed by assay (Fig. 2). Both normal and progeria cells showed a progressive prolongation of the clotting time after treatment with the specific immune globulins, but not with preimmune globulins or normal saline. This indicates that fibroblast TF is similar or identical to the placental TF used as antigen, and further, that both can stimulate clotting through the extrinsic mechanism.

It is not clear why cells from two genetically distinct disorders[6,7] show similar elevations in TF activity. Reports indicate that progeria and Werner's cells contain a diversity of altered proteins affecting HLA antigens, cytoplasmic enzymes[10,14–16] and insulin receptors[23]. Thus, although the TF of prematurely ageing cells seems to be recognised normally by specific antiserum, it may have a structural alteration such that a given amount possesses increased procoagulant activity.

An explanation is also needed for how an increased amount of procoagulant in prematurely ageing skin fibroblasts could relate to the severe vascular disease of progeria and Werner's syndrome[6,7]. Indeed, it would be extremely useful to know the TF activity and growth potential of fibroblasts from a second set of controls, subjects with severe atherothrombotic disease apart from progeria and Werner's syndromes. Such studies, now in progress, may confirm that increased procoagulant activity and impaired growth potential are important factors in atherothrombosis when they involve certain cells within the vascular tree[14]. In fact, there is evidence that the cultured 'fibroblast' may originate from vascular cells such as endothelium or pericytes[17,18]. The early concept of Rokitansky[19], still extant today[20,21], proposes that coagulation mechanisms acting in or on arterial walls contribute to atherosclerosis. Zeldis *et al.*[22] have found that plasma membranes of intimal and medial cells are rich in TF antigen, in large blood vessels and their vasa vasorum. Moreover, TF is particularly abundant in the vicinity of atherosclerotic plaques where it virtually encompasses cholesterol crystals. Thus, TF could initiate coagulation not only as a consequence of plaque disruption but also after minimal cellular damage[12,22]. In any case, it is possible that elevated TF in arterial cells is an important factor that predisposes individuals with progeria and Werner's syndromes to an increased risk of thrombosis, atherogenesis and premature death.

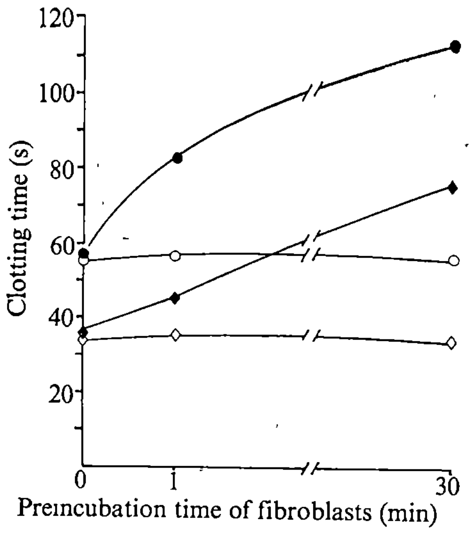

Fig. 2 TF activity of cultured fibroblasts incubated with and without specific immune globulins against purified TF. Fibroblasts were preincubated with immune globulins, preimmune globulins, or 0.9 NaCl and then assayed for TF. Early passage cells from a 22-yr-old normal male and from the 2.5-yr-old boy with progeria were used. ●, Normal cells+immune globulins; ◆, progeria cells+immune globulins; ○, normal cells+0.9% NaCl or preimmune globulins; ◇, progeria cells+0.9% NaCl or preimmune globulins.

We thank C. Lowery and E. Moerman for assistance and Dr Y. Nemerson for antiserum to TF. Supported by the NIH the MRC of Canada, the Canadian Diabetic Association Foundation Fund and the Ontario Heart Fund during the tenure of a MRC Scholarship to S.G.

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Thymic factor in human sera demonstrable by a cyclic AMP assay

NORMAL human serum has been reported to contain thymus hormone activity as detectable by a rosette inhibition assay[1]. It has been suggested that this circulating hormone may act through stimulation of cyclic AMP synthesis[2]. In addition, other thymic hormones isolated from the thymus, such as thymosin[3] and THF[4], have been found to activate membrane adenylate cyclase and to increase cellular levels of cyclic AMP in thymocytes[5,6]. Thymopoietin, a thymic hormone

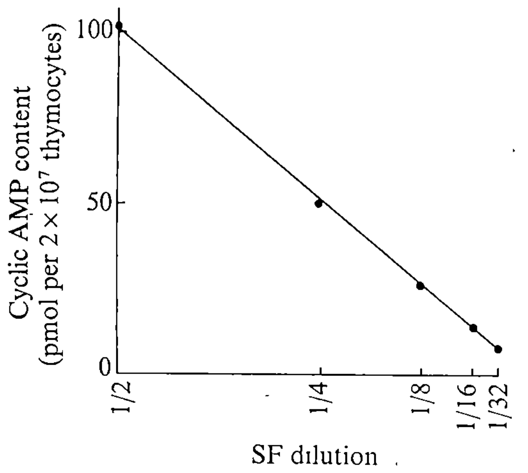

Fig. 1 Effect of different dilutions of SF on cellular cyclic AMP level. Human serum was collected by centrifugation at 4 °C after defibrination of the blood Serum was filtered on Amicon membrane CF50 (nominal cut-off molecular weight 50,000) by which high molecular weight thymic hormone inhibitors were removed[9]. Ultrafiltrate was added at different dilutions to Earle's solution containing thymocytes obtained from C57BL/6 mice aged 4–8 weeks. Cell concentration was adjusted to 2×10^6 cells ml^{-1}. After 5 min of incubation at 37 °C cellular cyclic AMP was extracted, placing tubes in liquid nitrogen for 1 min and subsequently in a boiling water bath for 5 min. Coagulated proteins were spun down in the cold and the supernatant directly assayed in duplicate, by Gilman's competition-binding procedure[10] with a cyclic AMP assay kit (TRK432, The Radiochemical Centre).

the amino acid sequence of which has been established[7], has also been claimed to induce T-cell differentiation by way of cyclic AMP[8] Here we report on a human serum factor (SF) capable of increasing the level of cellular cyclic AMP in thymocytes; we also present evidence for the possible thymic nature of this factor.

Figure 1 shows the effect of SF from a normal 18-yr-old donor on cyclic AMP levels in mouse thymocytes. Similar results were obtained with human thymocytes Among sera from different donors a marked variability in SF activity was observed. The differences in the increase of cyclic AMP levels were, however, in no way correlated with the cyclic AMP content of the sera (correlation coefficient=0.0304, $n=19$). In an attempt to establish the thymic origin of the SF we investigated sera from a total of 18 thymectomised myastenic patients (mean age 35.6 yr, s.d. 13.8). The SF activity was virtually absent in the thymectomised donors (mean 12.64 pmol cyclic AMP/2×10^7 cells, s.e. 1.96), whereas in normal healthy individuals ($n=399$; mean age 26.7 yr, s.d. 7.8) and in non-thymectomised myastenic patients ($n=5$; mean age 47 yr, s.d. 13.3) was consistently demonstrable (mean 76.69 pmol cyclic AMP/2×10^7 cells, s.e. 6.2; and mean 92.80 pmol cyclic AMP/2×10^7 cells, s.e. 21.81, respectively) When SF from normal donors was mixed with a similarly prepared material from thymectomised, no decrease in the activity was observed. The presence of SF inhibitors in the SF from thymectomised was therefore ruled out.

Table 1 Effect of adsorptions on SF activity

	pmol per 2×10^7 thymocytes
No SF added	12.8±0.8
SF (dilution 1:4)	61.8±2.5
SF (1:4) adsorbed on human thymocytes	11.0±1.4
SF (1:4) adsorbed on mice thymocytes	18.2±0.5
SF (1:4) adsorbed on human granulocytes	61.8±7.6
SF (1:4) adsorbed on mouse liver cells	88.4±0.6

Results are expressed as mean ± s.e of four experiments. SF was incubated for 1 h at 37 °C either alone or in the presence of 1×10^9 lyophilised human thymocytes or granulocytes per ml SF. When viable mouse thymocytes and liver cells were used for adsorption, the ratio of 4×10^8 cells per ml SF was used

We also investigated the binding capacity of SF to different cell types. To this end, adsorption experiments were carried out. It was found that SF activity could be completely adsorbed by lyophilised human thymocytes as well as by viable mouse thymocytes. On the other hand, adsorption with either lyophilised human granulocytes or viable mouse liver cells did not lead to a diminution of SF activity (Table 1).

Finally, the target specificity of SF and a number of agents known to elevate cellular cyclic AMP were tested on a panel of different mouse cells (Table 2). It seems therefore that SF is increasing cyclic AMP levels mainly in thymocytes with little or no effect on other lymphoid cells. In contrast, nonspecific stimulators of cyclic AMP, such as isoproterenol and prostaglandin E_1 (PGE_1), showed a marked stimulation in all target cells tested. Note that the increase in cyclic AMP in thymocytes given by the β-adrenergic activator isoproterenol could be selectively inhibited by the antagonist propranolol. Since the effect of SF is not inhibited to any extent by propranolol (Table 3), it seems that SF is acting through a membrane site, probably a receptor distinct from that for β-adrenergic activators. When isoproterenol and SF were added together, only a partial additive stimulation occurred. This indicates that, although they act on distinct membrane sites, isoproterenol and SF may stimulate the same adenylate cyclase regulatory subunit, as proposed for thymic hormones[8] The combination isoproterenol, propranolol and SF produced a similar increase in cyclic AMP as did SF alone, again indicating that the antagonism isoproterenol–propranolol did not interfere with

Table 3 Effects of SF, isoproterenol and propranolol on cyclic AMP level in thymocytes

	pmol per 2×10^7 thymocytes
No SF added	12.4±0.6
SF (dilution 1:4)	72.3±3.5
Isoproterenol (10^{-5}M)	220.9±1.0
Propranolol (10^{-5}M)	11.7±0.8
SF (1:4) + isoproterenol (10^{-5}M)	236.7±16.2
SF (1:4) + propranolol (10^{-5}M)	76.1±8.1
Isoproterenol (10^{-5}M) + propranolol (10^{-5}M)	10.5±0.1
SF (1:4) + isoproterenol (10^{-5}M) + propranolol (10^{-5}M)	77.5±2.3

Results expressed as mean ± s.e. of four experiments.

Table 2 Effects of SF and drugs on cyclic AMP levels in different target cells

	Thymocytes	Spleen cells	Lymph node cells	PBL*	Liver cells
No SF added	10.3±0.3	11.7±1.4	23.0±0.2	37.2±1.3	15.3±0.9
SF (dilution 1:2)	139.1±1.3	17.2±1.0	56.7±4.0	28.8±1.7	13.7±0.8
PGE_1 (10^{-6}M)	151.8±9.9	42.7±6.8	107.0±3.7	113.1±1.6	47.0±0.8
Isoproterenol (10^{-5}M)	218.8±1.1	46.5±9.3	83.1±2.0	81.4±1.7	37.4±1.3

Results expressed in pmol per 2×10^7 cells (mean ± s.e. of four experiments).
*Human peripheral blood lymphocytes

the capacity of the cells to respond to SF by an increased cyclic AMP level.

In conclusion, SF seems to be thymus derived, since the activity in thymectomised patients is absent. Furthermore, among the different target cells investigated, only thymocytes were found to possess a membrane site for SF in addition to the β-adrenergic receptor. Experiments are now being carried out to establish whether SF has the biological activity of the thymic hormone described by Bach *et al*[1]. The stimulation of cyclic AMP synthesis on thymocytes will eventually provide a quantitative and reproducible assay for the detection of human circulating thymic hormone.

A.A. is a European Molecular Biology Organization long-term fellow.

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Lack of an autosomal recessive genetic influence in vertical transmission of hepatitis B antigen

BLUMBERG *et al* have postulated that predisposition to the hepatitis B antigen (HBsAg) carrier state is inherited as an autosomal recessive gene[1]. Crucial to this concept, but lacking in Blumberg's populations, are families in which both parents are carriers. All offspring of such families should be homozygous for carrier predisposition and once infected all should become carriers. To test Blumberg's hypothesis, we compared rates of hepatitis B antigenaemia among children in two groups of families: those in which both parents were HBsAg carriers and those in which the mother was a carrier, but the father's serum was antibody (anti-HBs) positive. We did not include families when the father's serum was both HBsAg and anti-HBs negative, since carrier state predisposition remained unknown without evidence of previous hepatitis B virus (HBV) infection. If the autosomal recessive genetic hypothesis is correct, we would expect, in families with two HBsAg carrier parents, that all HBV-infected offspring would become carriers. In contrast, only a small proportion of children would become carriers in families with a carrier mother and an antibody-positive father, since most children would have only a single gene for carrier predisposition (inherited from their mothers) and would, like their fathers, form antibody after exposure rather than becoming carriers.

The families in this study were derived from a study of vertical transmission of the hepatitis B virus from carrier mothers in Taiwan to their newborn offspring. The details of the population studied and methods used have been published before[2,3]. Sera of newborns were tested periodically for HBsAg and anti-HBs, and other family members' sera were tested at least once. We have included here all families in which the mothers' sera contained HBsAg and the fathers' sera contained either HBsAg or anti-HBs. Data are included only on children who were at least 3 months old (when most babies who became carriers were first known to be HBsAg positive)[2].

Hepatitis B surface antigen was detected in all specimens by radioimmunoassay (RIA, AUSRIA-I™, Abbott Diagnostics)[4] and complement fixation (CF)[5,6]. Babies' sera were retested by the more sensitive AUSRIA-II™, so rates of antigenaemia among the babies are higher than described previously[2]. Antibody (anti-HBs) was detected by passive haemagglutination (PHA-Electronucleonics) with titres of 1 : 8 or greater considered positive[7].

The frequency of antigenaemia among the offspring (both the babies and their siblings) was unrelated to whether their father's serum was antigen or antibody positive (Table 1). Babies in both groups were followed from birth for the same time (mean, 12 months) and none of the babies or their siblings had clinical hepatitis. All but five of 27 babies retested after their first positive specimens (eight of nine with an antigen-carrier father and 14 of 18 with an antibody-positive father) remained HBsAg carriers (average of 11 months follow-up). One further baby in each group developed anti-HBs (neither had had previously detected antigenaemia). The geometric mean CF titre (GMT) for the mothers of the seven babies with either transient antigenaemia or antibody was 1 : 5.9±3.1 (± 1 s.d.), whereas the GMT for those who became carriers was 1 : 46.1±2.8 (± 1 s.d.). Only seven siblings (9.5%) had anti-HBs-positive sera (one of 27 with a carrier father and six of 47 with an antibody-positive father).

Blumberg's autosomal recessive genetic hypothesis for predisposition to the HBsAg carrier state has evoked considerable controversy[8]. The data from several large population studies are compatible with such an explanation for the observed carrier segregation[1,9–11]. Mazzur has reported retrospective evidence of vertical transmission among Blumberg's original populations[12]. These data, in spite of the evidence of vertical transmission, remain compatible with an autosomal recessive mode of inheritance for carrier predisposition, although again lacking two carrier parent families.

Such family clustering of HBsAg carriers could also be explained, as Lederberg and Petrakis pointed out, by the spread of an infectious agent through close personal contact, without any genetic influence[13,14]. Information from families with the critical matings of two HBsAg-carrier parents has been difficult to obtain, especially in countries with a low carrier prevalence. During a study of vertical transmission of HBV from antigen-carrier mothers to their newborn offspring in Taiwan (a high prevalence area), we found a large number of families with two antigen carrier parents. Data from these families, compared with those with a carrier mother and an antigen-negative, antibody-positive father, were incompatible with an autosomal recessive mode of inheritance. Whether the father was an HBsAg carrier or was antibody positive had no influence on the development of chronic antigenaemia in the newborn children or its existence in their siblings. The maternal CF titre, on the other hand, did influence the baby's response to HBV infection (transient antigenaemia, the development of anti-HBs, or a chronic HBsAg carrier state). In addition, we have reported previously that virtually 100% of babies and their siblings were chronic HBsAg carriers when the mother's serum had a very high CF titre (⩾1 : 64) (ref. 2).

Our findings suggest that all these children could become

Table 1 Hepatitis B antigenaemia among offspring of HBsAg carrier mothers: influence of HBsAg or anti-HBs status of fathers

Father	Babies No. of HBsAg positive/total*	Babies % Positive	Siblings No. of HBsAg positive/total*	Siblings % Positive
HBsAg positive	13/22	59.1	17/27	63.0
Anti-HBs positive	30/53	56.6	27/47	57.4

*$P > 0.5$ by χ^2.

HBsAg carriers, regardless of their genetic background. Development of the carrier state was probably influenced more by the dose of infectious virus they received (as suggested by the relationship between antigenaemia among the children and their mothers' CF titres). Schweitzer made a similar observation in his finding that vertical transmission from HBsAg carrier mothers in the USA only occurred when the mother's serum contained the e antigen (thought to be related to HBV infectivity)[15,16]. The early age of exposure in these children may also be important, since the age when infection occurs has been shown to influence the development of the carrier state in other settings[17]. Our data support this concept, since very few children had anti-HBs, indicating that when HBV infection occurred early in life, nearly all who were infected became carriers.

In summary, the data presented here seem to be incompatible with an autosomal recessive mode of inheritance for HBsAg carrier predisposition. It seems instead that the infectious dose and the age when infection occurs are more important determinants of the carrier state. The discrepancy between our findings and those of population studies may also indicate that family segregation analyses, especially of a contagious disease, cannot readily discriminate between the roles of environmental and genetic factors[18].

This study was supported through funds provided in part by the Naval Medical Research and Development Command, US Navy Department, and the Office of Naval Research.

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Human foetal pituitary peptides and parturition

THE pituitary gland produces a family of related peptide hormones (Fig. 1). Adrenocorticotrophic hormone (ACTH), β-lipotropic hormone (β-LPH) and possibly γ-lipotropic hormone (γ-LPH) are produced by the pars distalis. α-Melanocyte stimulating hormone (α-MSH), β-melanocyte stimulating hormone (β-MSH) and corticotropin-like intermediate lobe peptide (CLIP)[1], however, are thought to be formed in the pars intermedia[2], their being found only in those species in which this structure can be distinguished. They have not been identified in man[3]. The human foetus, however, does develop a rudimentary pars intermedia which involutes shortly after birth[4]. We present, for the first time, evidence for the occurrence of peptides resembling α-MSH and CLIP in the human foetal pituitary in the second half of pregnancy, and we suggest that these peptides are the dominant hormones of foetal life, only replaced by ACTH before parturition.

Human foetal pituitaries were dissected at autopsy following stillbirth, hysterotomy or neonatal death and adult human pituitaries obtained *post mortem*.

Fractionation of an adult pituitary extract on Sephadex G-100 showed two peaks of large molecular weight material reacting in the β-MSH assay and running in the expected positions of β- and γ-LPH (Fig. 2). There was no significant amount of low molecular weight material corresponding to the position of authentic (1–18) β-MSH. A substantial peak of material reacting in both the C- and N-terminal ACTH assays corresponded to intact ACTH, whereas a shoulder on the trailing edge of this peak contained C-terminal activity which could represent a small amount of CLIP. The final small peak of N-terminal activity can probably be attributed to an α-MSH-like peptide; the melanocyte-stimulating potency of this peak was greatly in excess of that in the ACTH peak but not as large as could be expected for authentic α-MSH.

Fractionation of a pituitary extract from a 24-week female foetus from whom the pituitary was dissected within one hour

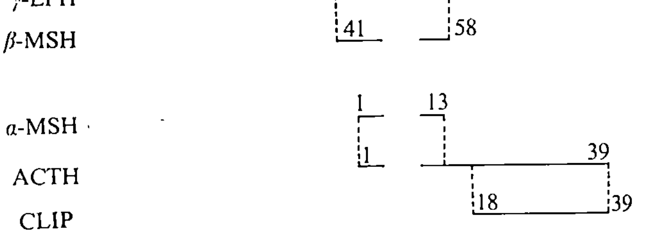


Fig. 1 Corticotropin, lipotropin and melanocyte stimulating hormones. β-LPH is a 91-amino acid peptide whose first 58 amino acids are identical in sequence to γ-LPH. β-MSH shares its 18-amino acid sequence with both β- and γ-LPH. ACTH is a 39-amino acid peptide whose first 13 amino acids are identical in sequence with α-MSH, and whose 18–39 sequence is identical to CLIP. · · ·, The common heptapeptide core responsible for melanocyte stimulating activity in β-LPH, γ-LPH, β-MSH, α-MSH and ACTH.

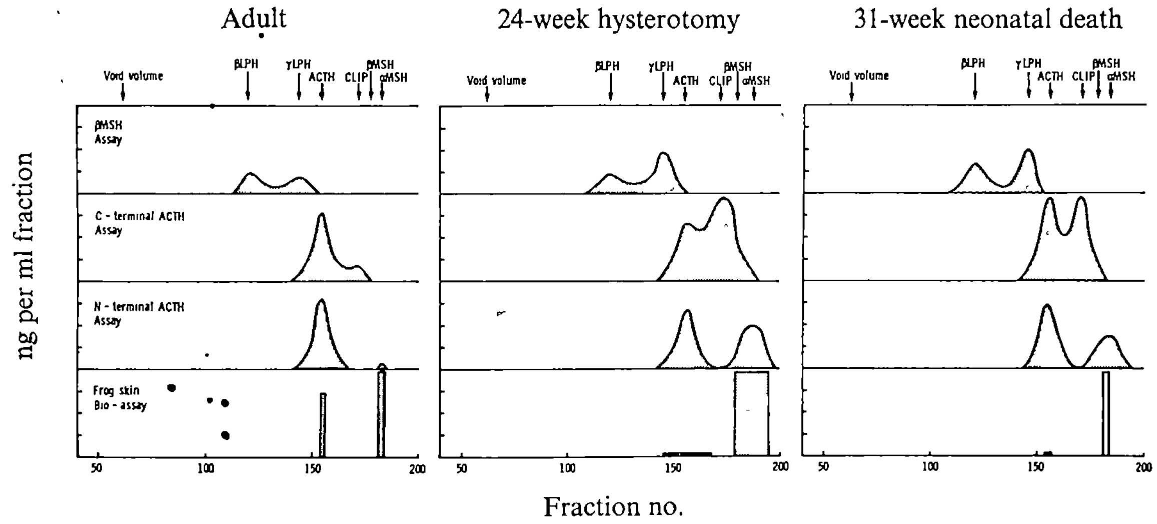


Fig. 2 Chromatography of pituitary extracts on Sephadex G-100. Pituitary fractionations from an adult, a 24-week foetus delivered by hysterotomy and a 31-week infant who survived 12 h post partum. Pituitaries were homogenised in 0.02 M HCl containing 10^{-3} M diisopropylphosphofluoridate. After centrifugation a sample from the supernatant was applied to a column (1.6 × 100 cm) of Sephadex G-100 and eluted in 1 M acetic acid. The fractions were analysed in three systems of radioimmunoassay: a β-MSH assay, a C-terminal ACTH assay and an N-terminal ACTH assay. The β-MSH assay[5] reacted with β-MSH, β-LPH and γ-LPH. The C-terminal ACTH assay[6] reacted with ACTH and CLIP. The N-terminal ACTH assay[7] reacted with ACTH and α-MSH. Selected fractions encompassing the peaks for α-MSH and ACTH were also examined for melanocyte stimulating activity by an adaptation of the frog skin bioassay[8]. In this assay the relative potency of α-MSH to ACTH is 1,000:3. Arrows indicate expected positions of β-LPH, γ-LPH, ACTH, CLIP, β-MSH and α-MSH for each of the chromatograms.

of hysterotomy also showed large molecular weight material reacting in the β-MSH assay representing β- and γ-LPH (Fig. 2). As with the adult there was no evidence of low molecular weight authentic β-MSH. A peak of material reacting in both the C- and N-terminal ACTH assays corresponded to intact ACTH. This, however, was followed by a more substantial peak of material which contained solely C-terminal activity and a significant peak of low molecular weight N-terminal activity. The biological potency of the latter was again far in excess of that which could be accounted for by ACTH.

A pituitary extract from a 31-week male infant who died 12 h after a normal delivery was fractionated and demonstrated a pattern intermediate between that of the adult and that of the 24-week foetus (Fig. 2). The peak of material in the C- and N-terminal ACTH assays which corresponded to intact ACTH was approximately equal to the peak of material with solely C-terminal activity and exceeded the peak of low molecular weight N-terminal activity. This last peak again displayed high biological potency. There were peaks representing β- and γ-LPH as before, but no suggestion of any activity that could represent β-MSH.

Extracts of a further four foetal pituitaries were analysed on a BioGel P6 column which gives a clear distinction between ACTH and CLIP. Two stillborn foetuses at 33 and 38 weeks showed a striking preponderance of C-terminal activity over intact ACTH (Fig. 3). In this they resembled the 24-week foetus delivered by hysterotomy (Fig. 2). The proportion of intact ACTH, however, was much increased in a full-term stillborn foetus and was the dominant hormone in a full-term infant who survived 5 h. In two adult pituitaries (69 and 71 yr) there was a large preponderance of ACTH over material resembling CLIP.

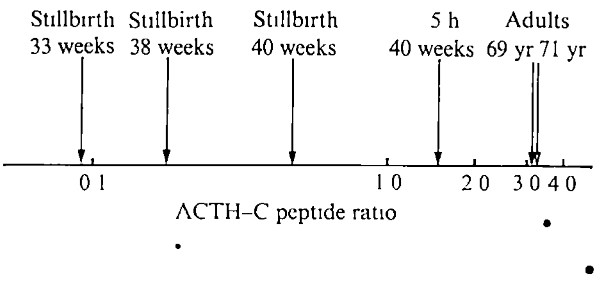
Since the 31-week premature infant who survived 12 h (Fig. 2) resembles the two full-term foetuses rather than those of 33 and 38 weeks, it may be that the changing ratio of the small C-terminal peptide to intact ACTH seen in Fig. 3 is associated with parturition rather than with advancing gestational age.

These studies demonstrate that for the most part of intrauterine life the human foetal pituitary contains only small amounts of intact ACTH, but relatively larger quantities of peptides closely resembling α-MSH and CLIP. It seems probable that the presence of these small molecules reflects a physiological function in the foetus which is not present in postnatal life. It is of interest to consider this finding in the light of other information on the development of pituitary–adrenal function in the human foetus.

The foetal zone of the human adrenal cortex achieves great size during gestation. At parturition it undergoes rapid involution and the definitive zone hypertrophies. It is commonly argued[9,10] that foetal pituitary ACTH is the trophic hormone for both the foetal and definitive zones of the adrenal cortex. ACTH administered exogenously, however, is not itself capable of stimulating the foetal zone[11], and our findings suggest that ACTH is not produced in large quantities by the human foetal pituitary during the major part of gestation. That the trophic hormone responsible for the growth of the foetal zone might be one of the small peptides identified here, for the first time, is an attractive hypothesis. We have demonstrated that near parturition there is a sharp increase of intact ACTH relative to the smaller peptides. If this were reflected in plasma concentrations the metamorphosis of the foetal adrenal gland during this period would be explicable. The transference of function from foetal to definitive zone would accompany the switch in peptide synthesis by the pituitary.

It is the physiological significance of these events, however, which is of most interest. Liggins has demonstrated that the key event preceding parturition in sheep is a surge of foetal cortisol from the foetal adrenal cortex[12]. This event not only helps prepare the foetal lamb for extrauterine existence (for example the production of surfactant in the foetal lung) but it is itself the

Fig. 3 Ratio of ACTH activity to small C-terminal peptide activity in adult and foetal pituitaries as assessed by chromatography on BioGel P6. The foetal pituitaries were obtained from a 33-week stillbirth, a 38-week stillbirth, a 40-week stillbirth and a full-term infant who survived 5 h. Adult pituitaries were obtained *post mortem* from patients aged 69 and 71 yr.



trigger for the initiation of parturition. Premature delivery can in fact be induced at any time in the second half of pregnancy by infusing the foetal lamb with ACTH or cortisol.

It is impossible to determine by direct experiment whether the production of cortisol is of similar significance in the human foetus. Cord blood values for cortisol are, however, greater in babies delivered spontaneously than in those delivered by induction or Caesarean section[13]. It has also been demonstrated that the levels of cortisol in amniotic fluid remain low until week 34 of gestation, but then increase sharply until term[14].

The foetal zone of the human adrenal cortex, though highly active in steroidogenesis, is unable to synthesise glucocorticoids. It is therefore probable that the increased cortisol production derives from the definitive zone. If our hypothesis proves correct that one or other of the small peptides resembling α-MSH and CLIP are the trophic hormones for the foetal zone, while intact ACTH drives the definitive cortex, then the increase of ACTH relative to the small peptides which we have described near parturition would seem to be functionally related to the increasing production of cortisol by the definitive zone of the foetal adrenal gland. Thus the switch in peptide synthesis may be the central mechanism in the chain of events that leads from the passive placenta-dependent foetus to the viable responsive newborn baby.

We thank Drs R. F. Alexander, M. S. Ross, Morag Ellison, Sylvia Lawler, Professor P. Huntingford, Mr A. J. Woolf and the staff of the tissue bank of the Royal Marsden Hospital for the provision of foetal pituitaries, and Dr A. P. Scott for most valuable advice regarding the extraction procedure R.E.S. was supported by the Wellcome Trust and is at present an MRC Research Fellow.

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Absolute identification of melatonin in human plasma and cerebrospinal fluid

MELATONIN, the compound responsible for the lightening of amphibian skin, was extracted from bovine pineal glands and chemically characterised by Lerner *et al.*[1,2]. Recent reviews[3–5] have suggested that melatonin has an endocrine role, particularly in relation to chronobiology, pituitary function and cerebral physiology. Most of this work has been accomplished by animal experimentation So far the evidence for the existence of melatonin in man has been indirect. It is formed biosynthetically from tryptophan and the necessary enzyme system is known to be present in the human pineal throughout life[7]. There is however little evidence to suggest that it circulates in human blood or cerebrospinal fluid (CSF). Attempts to identify melatonin by bioassay[8] or radioimmunoassay[9] have not been accompanied by the chemical identification of the substance measured. We present here, for the first time, unequivocal evidence based on gas chromatography (GC) and mass spectrometry (MS) of the presence of melatonin in human plasma and CSF.

Plasmas from normal volunteers and CSF from patients undergoing lumbar puncture as part of other clinical investigations were used. Individual samples were extracted with chloroform. Most lipid and pigment was removed from the chloroform extract by silica gel column or thin-layer chromatography with hexane–ether (80 : 20) as solvent. Melatonin was eluted with methanol and blown to dryness under argon. Using tritiated melatonin, recoveries in excess of 80% were found at this stage.

Synthetic melatonin was treated with a specific silanising reagent (Sylan BTZ, Supelco) to form the indole *N*-trimethylsylil derivative. When a Varian Mat 311A mass spectrometer interfaced to a Varian Aerograph 1400 GC was used, the mass spectrum of this derivative gave rise to two major fragments at mass to charge ratio (m/e) 232 and 245 and a molecular ion at m/e 304 of lower intensity (Fig. 1).

Plasma and CSF extracts were silanised similarly. Pooled plasma extracts (3-ml equivalents) yielded a component with GC retention time and mass spectrum identical with those of synthetic melatonin (Fig. 1) Individual plasma and CSF extracts equivalent to 0.2 ml were subjected to high resolution mass fragmentography at m/e 232 and 245 to give, at the correct GC retention time for melatonin, peaks with accurate mass identical to those of synthetic melatonin.

The elucidation of any physiological or even pathological role for melatonin in man requires first its identification and subsequently its quantification. Our results constitute the first absolute identification of melatonin in human plasma and CSF. Moreover, the major ions 232 and 245, since they carry a large proportion of the total ion current of silanised melatonin, may be utilised in a single ion detection procedure for sensitive quantitative analysis. Thus GCMS in demonstrating the existence of circulating melatonin in man provides at the same time a method for its assay

P.E.M. and R.E.S. are supported by MRC research fellowships.

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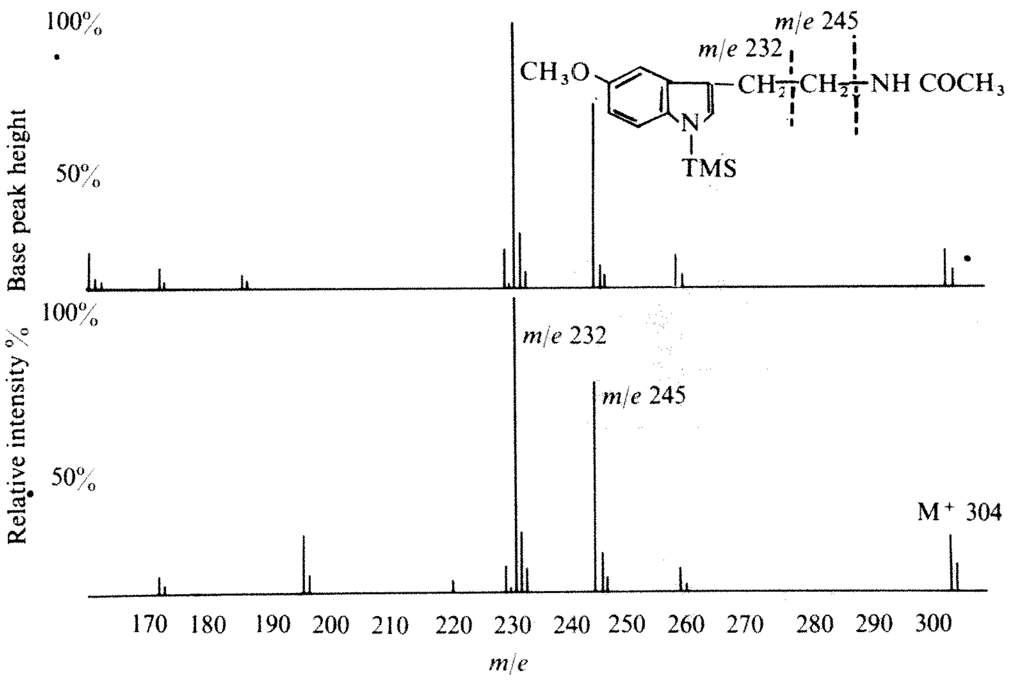

Fig. 1 Partial mass spectra obtained from silanised synthetic melatonin and silanised plasma extract. The bonds which fragment to give rise to the two major ions are indicated.

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Protection of spermatogenesis by α_{2u} globulin in rats treated with oestrogen

α_{2u} GLOBULIN is an androgen-dependent rat urinary protein with a molecular weight of about 23,800 (ref. 1). In the male rat, starting from puberty until senescence, α_{2u} globulin is synthesised and secreted by the hepatic tissue and is rapidly cleared by the kidneys into the urine[2–4]. In addition to its urinary concentration α_{2u} globulin has been found to be concentrated by the salivary glands and released into the saliva (A.K.R., and J.G.B., in preparation). Synthesis of this protein could be induced in the spayed female rat by androgen, and oestrogenic compounds completely inhibited α_{2u} synthesis in the mature male rat[5]. But α_{2u} globulin does not bind testosterone, oestradiol-17β or any of their metabolites (unpublished results of A.K.R.). Pretreatment of mature male rats with oestrogen induces a dose-dependent temporary lag period within which α_{2u} globulin is not synthesised even under androgenic stimulation. With a daily oestradiol dose of 50 μg per 100 g body weight a lag period extending up to 3–4 weeks has been established[6]. Although the adult male rat synthesises 20–30 mg of α_{2u} globulin per day, no physiological role of this protein has yet been established. The close parallel between the male reproductive capability and the production of α_{2u} globulin[4,5] prompted us to look into the possible reproductive role of this protein. The discovery of the oestrogen-mediated lag period in the mature male rats also provided an opportunity to look into the spermatogenic abnormalities within this lag period and any reparative effects which α_{2u} globulin may have on such damage. The results reported here demonstrate considerable protection of spermatogenesis by α_{2u} globulin against potential damage by oestrogen.

α_{2u} Globulin was prepared from male rat urine as before[7]. The protein was purified further on a Sephadex G-75 column. α_{2u} Globulin prepared according to the above procedure gave a single electrophoretic band on sodium dodecyl sulphate(SDS)–polyacrylamide gel electrophoresis. Because investigations by Lacy and Lofts[8] have indicated that damage to spermatogenesis caused by oestrogen could be reversed by follicle stimulating hormone (FSH), it was considered important to rule out any possible trace of contamination of FSH in our α_{2u} preparation.

Potent antiserum against FSH-B was prepared by repeated injection of the hormone with Freund's complete adjuvant into rabbits, using a procedure outlined for preparation of antiserum against α_{2u} globulin[7]. The antiserum prepared according to the above procedure, when tested on Goodman plates[9], produced four precipitin lines against 25 μg of FSH-B and three precipitin lines (one major and two minor) with the same amount of FSH-1. Even 100-fold excess of α_{2u} globulin (2.5 mg) showed no precipitin reaction against the antiserum. Double antibody radioimmunoassay[10] of a solution (2.5 mg ml^{-1}) of α_{2u} globulin preparation under various dilutions with the anti-FSH and FSH-1 system also failed to detect any trace contamination of FSH in the α_{2u} preparation. Antiserum prepared against α_{2u} globulin also yielded no precipitin reaction when tested against whole pituitary homogenate.

Mature male rats of Yale strain (about 100 d old at the start of the experiments) were used for this investigation. Thirty-five animals were treated daily with oestradiol-17β for 7 d (50 μg per 100 g body weight per d). At the end of treatment, five animals were killed and their testes were fixed in Bouin's fixative and used for paraffin embedding. The remaining 30 animals were divided into two groups, one of which received injections of 0.2 ml of 0.14 M NaCl per day and the other received subcutaneous injections of α_{2u} globulin (1 mg in 0.2 ml of 0.14 M NaCl per animal per day) for the rest of the experimental period. Five animals from each group were killed at 7-d intervals and their testicular tissue was fixed in Bouin's fixative and used for

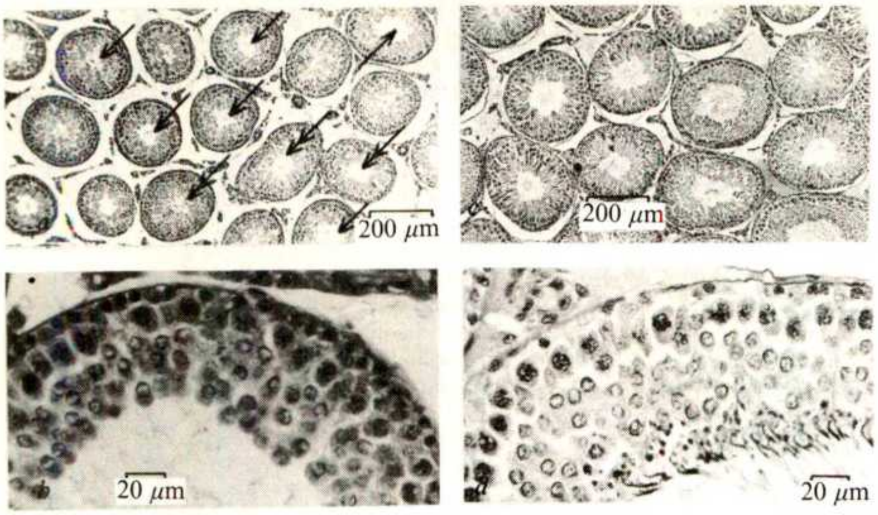

Fig. 1 Photomicrographs of PAS–haematoxylin-stained sections of the testes from animals treated with oestrogen alone and with oestrogen followed by α_{2u} globulin. *a*, Testicular sections from control animals receiving daily injections of oestradiol-17β for the first 7 d followed by injections of 0.2 ml of 0.14 M NaCl (placebo) per day for the next 3 weeks. Tubules marked with → show absence of step 15 to step 19 spermatids. Tubules marked with →→ show drastic reduction of step 9 to step 14 spermatids. Considerable shrinkage of the tubular diameter could also be noted when compared with (*c*). *b*, Treatment same as for (*a*). This photomicrograph shows the cross section of a tubule at stage VIII of the seminiferous cycle. Note the absence of step 19 spermatids. *c*, Testicular section from an animal treated daily with oestradiol-17β for the first 7 d, followed by daily treatment with α_{2u} globulin for the next 3 weeks. The tubular sections show normal spermatogenesis with normal cellular associations at various stages of the cycle. Unlike the section shown in (*a*), no tubular shrinkage could be seen. *d*, Treatments same as for (*c*). This photomicrograph shows the cross section of a tubule at stage VIII of the seminiferous cycle. In contrast to (*b*) the tubule shows normal spermatogenesis proceeding up to mature step 19 spermatid.

paraffin embedding. Sections (5 μm thick) of the testes were stained with periodic acid–Schiff (PAS)–haematoxylin and used for histological evaluation[11,12].

The stained sections from animals treated for 7 d with oestradiol-17β and killed on the eighth day showed no discernible change in spermatogenesis. Testicular sections from animals treated for 7 d with oestradiol and another week of rest period (no hormone treatment) also showed almost normal morphology. Tubular shrinkage and degenerative changes were found to appear at the second week after the last oestradiol treatment. Seminiferous epithelium in these groups of animals (2 weeks after last oestradiol treatment) showed drastic reduction and in some sections complete absence of spermatids of step 9 to step 19. Some of the pachytene spermatocytes within stages VIII through XIII also showed degenerative changes. The testicular sections from animals killed 3 weeks after the last oestradiol treatment showed relatively small and atrophic tubules. Qualitatively, spermatogenesis was found to progress only up to step 7–8 spermatids. Step 9–19 spermatids were found to be absent in most of the tubules and in some instances when present were invariably abnormal (Fig. 1*a* and *b*). Quantitative evaluation of the abnormality within the various stages of the seminiferous cycle are summarised in Table 1.

Table 1 Quantitative evaluation of the oestrogenic damage and its prevention by α_{2u} globulin of the seminiferous epithelium at various stages of the spermatogenic cycle

Cellular associations (stages of the cycle)	Index of degeneration in group 1 (oestrogen alone)			Index of degeneration in group 2 (oestrogen followed by α_{2u} globulin)		
	Day 14	Day 21	Day 28	Day 14	Day 21	Day 28
I	0	80	72	0	0	0
II	0	25	58	0	0	0
III	0	62	73	0	0	0
IV	0	66	100	0	0	0
V	0	55	90	0	0	0
VI	6	42	93	0	0	0
VII	7	82	100	0	0	0
VIII	0	50	100	0	0	0
IX	8	83	19	0	0	0
X	0	83	11	0	0	0
XI	0	54	16	0	0	0
XII	4	72	42	0	0	0
XIII	0	72	42	0	0	0
XIV	0	40	31	0	0	0

All animals were treated for the first 7 d with oestradiol-17β (50 μg in 0.1 ml vehicle per 100 g body weight per day; subcutaneous injection of the hormone as an emulsion in 0.1 M sodium phosphate, *p*H 7.2, propylene glycol and Tween 80, 89.6:10.0:0.4, by volume). After initial oestrogen treatment, the animals in group 1 received no other treatment except daily injections of 0.2 ml of saline (0.14 M NaCl). Beginning from the 8th day (1 d after the last oestrogen injection) until death, the animals in group 2 received daily injections of α_{2u} globulin (1 mg per rat per d, subcutaneously in 0.14 M NaCl). The tubular cross sections showing the presence of degenerating germinal cells and/or containing less than 50% of any particular cell type within the specific cellular association were considered to be abnormal. Percentage tubular cross section showing abnormality is used as the index of degeneration.

$$\text{Index of degeneration} = \frac{\text{No. of abnormal tubular cross sections}}{\text{Total no. of tubular cross sections examined}} \times 100$$

The fractional numbers are brought to the nearest whole number.

The group of animals given daily injections of α_{2u} globulin after initial treatment with oestradiol-17β showed no degenerative changes in their testicular sections throughout the period of investigation. The testicular sections from this group of animals 1, 2 and 3 weeks after the last oestradiol treatment showed an intact seminiferous cycle leading to normal spermatogenesis (Fig. 1*c* and *d*; Table 1).

In another series of experiments, adult male rats after the first 7 d of oestrogen treatment were subsequently treated with testosterone alone (250 μg per 100 g body weight) and testosterone plus α_{2u} globulin. Although testosterone, together with α_{2u} globulin, could provide complete protection against the oestrogen-induced damage to spermatogenesis, testosterone alone failed to do so.

The results presented here show that α_{2u} globulin can provide protection against oestrogen-mediated damage of spermatogenesis in the rat. We have shown before that oestrogen treatment completely stops the synthesis of α_{2u} globulin within 7 d, and with a dose of 50 μg per 100 g body weight reinitiation of α_{2u} synthesis does not start until 3–4 weeks later[6]. Therefore, any possible spermatotropic role of α_{2u} globulin is expected to be missing within the oestrogen-mediated lag period. The observed damage to spermatogenesis, in particular the failure of spermatid maturation in the oestrogen-treated group, and the prevention of such inhibitory effect by α_{2u} globulin within the lag period, may indicate a physiological role of α_{2u} globulin in spermatogenesis.

We thank Dr V. N. Reddy, Dr N. J. Unakar and Marian Wilson for criticisms and suggestions. Rat FSH and anti-FSH preparations were given by the NIAMDD rat pituitary hormone distribution program of the National Institute of

Arthritis, Metabolism and Digestive Diseases. This work was supported by a grant from the National Institutes of Health

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Biochemical responses in lungs of ozone-tolerant rats

THE health hazard of ozone, the principal oxidant air pollutant of photochemical smog, has been well documented[1–3]. One of the notable features of ozone toxicity is the development of tolerance. When animals are exposed to a single sublethal dose of ozone they become resistant to subsequent lethal exposure. This tolerance phenomenon has been demonstrated in several species, and the effect has been shown to persist for well over one month[3–8]. One of its toxic effects has been attributed to its oxidative nature, and, more specifically, the initiation of lipid peroxidation[7–9]. Glutathione peroxidase (GP), which uses the reducing equivalent of reduced glutathione (GSH) to catalyse the decomposition of toxic hydroperoxides, and metabolically related enzymes glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD), have been suggested as a key mechanism for protection of cellular components from deleterious effects of hydroperoxides[10,11]. The activities of these enzymes have been found to increase in the lungs of rats exposed to chronic levels of ozone[9], and in tissues of rats subjected to other oxidative stress[12,13] Thus changes in the activities of these enzymes seem to be related in some way to the defence mechanism against peroxidative damage. In this study, rats tolerant to ozone maintained higher activities of GP, GR and G6PD, and higher levels of GSH in the lungs following excessive exposure. The adaptive response of this potentially protective enzymic system in the lungs of ozone-tolerant rats may therefore be partly responsible for their resistance against lethal exposure.

Seventy-day-old male Sprague–Dawley rats (chronic respiratory disease free) were initially exposed to either 0 or 0.76 p.p m. ozone for 3 d (to develop tolerance) in monitored chambers as described previously[9]. After 8 d of recovery in an air-filtered room, portions of tolerant and non-tolerant animals were exposed to either 0 or 4.0 p.p.m. ozone for 8 h Sixteen hours later surviving animals and their respective controls were killed by exsanguination following pentobarbital injection. After perfusing with saline through the pulmonary artery to remove blood, the lungs were homogenised, fractionated and assayed for the activities of GP, GR, G6PD, and levels of protein and GSH as described previously[9,14]

Sixteen hours after the end of the acute exposure, five out of ten non-tolerant rats died compared with only one out of seven of the tolerant group. The lungs of both acute exposed groups exhibited severe haemorrhage and oedema, but the tolerant animals showed less severe injury. The biochemical responses of the lungs are summarised in Table 1. Exposure of rats to 4.0 p.p.m. ozone for 8 h resulted in a marked increase in protein content, and a significant ($P<0.05$) decrease in the activity of GP, GR and G6PD, and levels of GSH in the lungs of both tolerant and non-tolerant groups. Tolerant animals, however, showed significant, higher activities (from Student's *t* test) of GP, GR and G6PD, and higher GSH levels in the lungs than

Table 1 Biochemical responses in the lungs of rats exposed to ozone 16 h after the end of exposure to 4 p.p.m. ozone for 8 h

Biochemical parameter	Tolerance*	Exposed (mean ± s.d.)	Control	*P*
Protein (mg per lung)	Yes	136 ± 6	71 ± 4	<0.001
	No	152 ± 17	73 ± 3	<0.001
		P NS	NS	
Reduced glutathione (nmol non-protein sulphydryl per mg protein)	Yes	15 ± 2	31 ± 2	<0.001
	No	11 ± 1	29 ± 4	<0.001
		P <0.01	NS	
Glutathione peroxidase[8] (nmol NADPH oxidised or reduced per min per mg protein)	Yes	42 ± 3	67 ± 5	<0.001
	No	34 ± 4	64 ± 3	<0.001
		P <0.01	NS	
Glutathione reductase[8] (nmol NADPH oxidised or reduced per min per mg protein)	Yes	22 ± 3	40 ± 5	<0.001
	No	18 ± 2	39 ± 2	<0.001
		P <0.02	NS	
Glucose-6-phosphate dehydrogenase[8] (nmol NADPH oxidised or reduced per min per mg protein)	Yes	44 ± 4	60 ± 5	<0.001
	No	37 ± 4	59 ± 3	<0.001
		P <0.02	NS	

*Exposed to 0.76 p.p.m. ozone for 3 d and allowed to recover for 9 d. NS, Not significant ($P \geq 0.05$).

those of the non-tolerant rats. When the data are expressed as total units per lung, tolerant rats still show a higher level of GSH and higher activities of GP, GR and G6PD. It should be noted that the above biochemical parameters in the lungs of rats recovering for 9 d from the initial exposure were not different from those of the non-exposed controls (Table 1)

In another experiment, 60-d-old male rats were initially exposed to either 0 or 0.73 p.p m. ozone for 3 d. Eleven days after exposure, 11 tolerant and 13 non-tolerant animals were exposed to 3.9 p.p.m. ozone for 8 h Only one of the tolerant rats had died 18 h later, as against seven of the non-tolerant group. The lungs of tolerant animals also showed relatively less severe injury and maintained higher activities of GP, GR and G6PD, and higher GSH levels.

Throughout this research, it was observed that the activities of GP, GR and G6PD, and GSH levels in the lungs of animals exposed to 0 8 p.p.m. ozone for 3 d increased significantly above those of the controls, and that these biochemical changes began to revert towards control values in 2 d, reaching control levels about 7 d after recovering in ozone-free air (unpublished observations). Even though the biochemical changes in the lungs of tolerant rats completely returned to control level, significantly higher activities of GP, GR and G6PD, and GSH levels, accompanied by a reduced mortality rate and lung injury were developed when this group of rats were subjected later to the acute exposure. Although the precise significance and specificity of this correlation remain to be seen, it is possible that higher activities of GP, GR and G6PD, and GSH levels in the lungs of tolerant animals may render them more resistant to peroxidation damage induced by ozone. It has been shown that ozone-tolerant animals maintained higher GSH levels in the lungs, and theorised that the activities of pentose shunt pathway may be related to resistance or tolerance to injury from inhaled irritants[4,15].

The mechanisms of the differential biochemical response between the tolerant and non-tolerant rats to acute ozone exposure remain to be elucidated. It is possible that the enzymic synthesis machinery in the lungs of tolerant rats remained 'potentially active' even though the enzyme activities reverted to control levels. It has been shown morphologically that ozone primarily affects the type 1 cells of small conducting airways and adjacent alveoli[16,17]. Following injury to less than 1 p p m. ozone, repair processes, as evidenced by the replacement of type 1 cells by the cuboidal epithelial cells in the injured areas, begin within 24 h and reach a maximum between 48 and 72 h When animals are allowed to recover in room air, the cuboidal cells are gradually transformed into type 1 cells, and the tissue returns to normal in about 7 d (refs 16 and 18) From a morphological point of view, therefore, the newly transformed type 1 cells may be potentially more prone to metabolic change and thus become more resistant to subsequent exposure. Further studies such as morphometric measurement of type 1 and type 2 cells are, however, needed to clarify this view.

The GP system also has an important role in protecting red blood cells against oxidative damage. When red cells genetically lack one or more of these enzymes or enzymes involved in GSH synthesis, they become susceptible to oxidative drugs which induce haemolytic anaemia[19,20] The GP system may also be involved in tolerance phenomena induced by other types of oxidative agents, as GP uses a variety of hydroperoxides as substrates[10].

The present observations that tolerant animals are capable of maintaining higher activities of GP, GR and G6PD, and GSH levels in the lungs suggest that this enzymic system may be partly responsible for their resistance against lethal ozone exposure This study provides evidence for the possible role of the protective GP system in the development of tolerance to oxidative damage.

This work was supported by research grants from the US Public Health Service.

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Received December 22, 1975, accepted February 24, 1976.

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Mutagenic action of ascorbic acid

MICROBIAL[1], mammalian[2,3] and human[4,5] bioassays are being applied to uncover the mutagenic action of synthetic compounds which have been introduced accidently or intentionally into the environment Somewhat less attention has been given to the potential genetic hazard of chemicals which are an essential part of human nutrition or are formed within mammalian tissues and organs. Ascorbic acid belongs to this group of "neglected" naturally occurring compounds, although its capacity to form radicals and its interaction with viral[6] and bacterial[7] DNA should have raised suspicion about its possible mutagenic capacity. Considering the large daily intake of ascorbic acid, its addition to many food products[8] and its potential use as an inhibitor of the intragastric formation of *N*-nitroso compounds[8,9] and nitrosamine formation in food products[10], its action on the genome of human cells requires investigation. We here present evidence, from several experimental systems, suggesting that vitamin C, particularly in the presence of copper, has a mutagenic effect.

Several endpoints were used to uncover the effect of freshly prepared ascorbic acid and an ascorbic acid–Cu^{2+} mixture on the genome of cultured human fibroblasts. DNA fragmentation was measured as a shift in sedimentation profile after centrifugation in an alkaline sucrose gradient[11], DNA repair was estimated as unscheduled uptake of tritiated thymidine (^3H-TdR) into non-dividing cell populations[13] and chromosome aberrations were quantified by counting breaks and exchanges on the first metaphase plate after treatment Fibroblasts were obtained from small skin biopsies of four healthy 20–26-yr-old females and males and were used at the third to fifth tissue culture passage. The mutagenic capacity of ascorbic acid and an ascorbic acid–Cu^{2+} mixture were tested further using Ames' tester strain TA 100 of *Salmonella typhimurium*[1].

Cultured human fibroblasts treated with a mixture of ascorbic acid (Mallinckrodt) and Cu^{3+} exhibited DNA fragmentation (Fig. 1), DNA repair synthesis (Fig 2), and chromosome aberrations including chromatid breaks and exchanges (Table 1). An application of $CuSO_4$ at concentrations varying from 10^{-6} to 10^{-4} M had no detectable effect

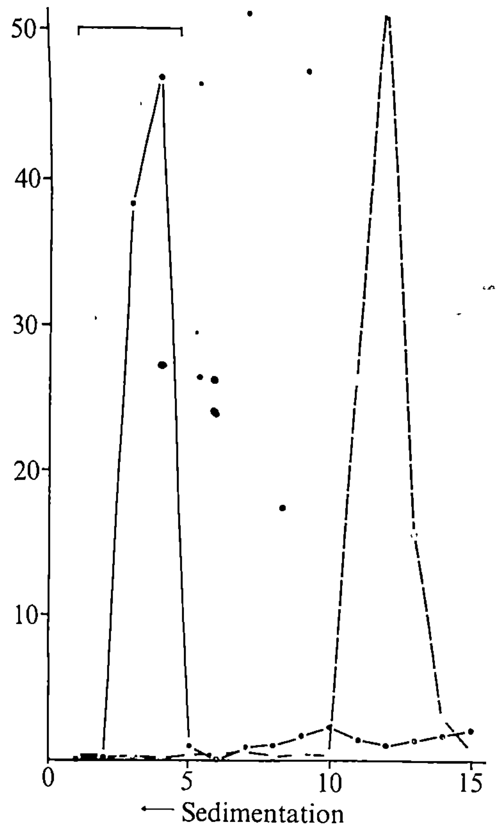


Fig. 1 Alkaline gradient sedimentation profiles of ^{3}H-DNA released from cultured human fibroblasts. ●, Cells exposed for 2 h to 1.8×10^{-5}M $CuSO_4$; ○, cells exposed for 2 h concurrently to ascorbic acid (1.8×10^{-3}M) and $CuSO_4$ (1.8×10^{-5}M). Horizontal bar indicates the sedimentation range into which DNA from untreated fibroblasts falls ^{3}H-TdR (1 µCi ml^{-1}) was added to the replicating fibroblasts for 20 h; thereafter the cell cultures were exposed to the compounds, rinsed four times with cold 0.024 M EDTA/0.075 M NaCl buffer (pH 7 5), scraped from the Petri dish surface, pelleted (3 min, 1,000 r p.m.), resuspended to give 10^4–10^6 cells per 100 µl, and placed on a lysing solution[11] on top of a linear alkaline sucrose gradient (5–20%, 5 ml) which was prepared over a 1-ml cushion of 2.3 M sucrose. After a 10-min lysis period at 25 °C the gradients were centrifuged at 25,000 r.p.m. for 30 min.

Table 1 Metaphase plates of cultured human fibroblasts with chromosome aberrations (breaks and exchanges) after a 2-h exposure to oxidised ascorbic acid and an ascorbic acid $CuSO_4$ mixture

	Metaphase plates with chromosome aberrations (%)*	
	Ascorbic acid+O_2†	Ascorbic acid+Cu^{2+}‡
2×10^{-3} M	25.8	—§
1×10^{-3}	28.5	—
6×10^{-4}	23.4	—
3×10^{-4}	15.0	26.5
1.5×10^{-4}	5.0	5.4
Controls	0–15	

Controls were cultured cells untreated with the ascorbic acid preparations.

*Samples were taken 18 h after treatment and 4 h after application of colchicine.

†O_2 was flushed through the ascorbic acid solution for 2 h before addition to the cultured fibroblasts.

‡The ascorbic acid–$CuSO_4$ ratio was 1,000:1

§Concentrations of 6×10^{-4}M and greater suppressed cell division.

Table 2 Frequency of mutations (*his*$^+$ revertants) of *S. typhimurium* (TA 100) after exposure to ascorbic acid, ascorbic acid plus Cu^{2+} or MNNG

	his$^+$ Reverents per 10^6 survivors*		
	Ascorbic acid	Ascorbic acid+Cu^{2+}	MNNG
3.5×10^{-2}M	2.2	160.0	—
2.5×10^{-2}	1.4	108.7	3,090.9
1.5×10^{-2}	0.7	3.8	—
1.0×10^{-2}	1.6	1.2	—
5.0×10^{-3}	1.1	1.0	171.4
Control†	1.1 (0.5–2.1)	—	—

*Bacteria in suspension were treated with the various compounds for 15 min at 22 °C, washed, centrifuged, resuspended with nutrient broth and plated on histidine-deficient culture medium[1]. The ascorbic acid+$CuSO_4\times5H_2O$ (1,000:1) solution was incubated for 30 min at 37 °C before use

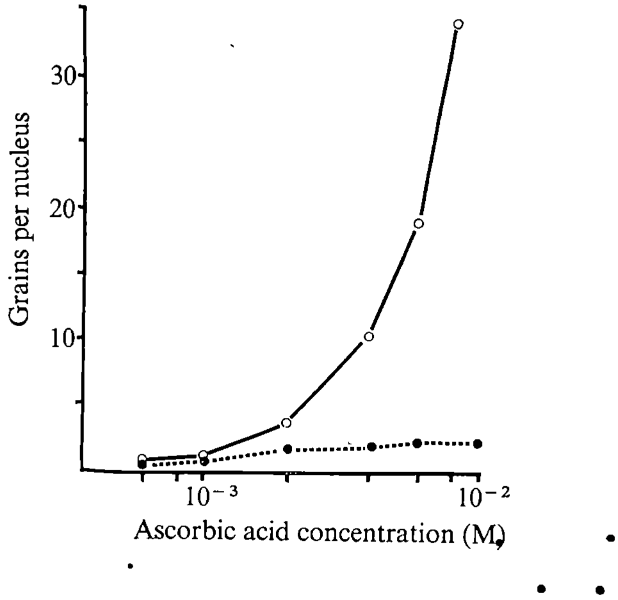
†Controls were bacteria untreated with the various chemical preparations. The range of mutation frequencies is shown in parentheses.

on DNA and chromosomes. If the ascorbic acid solution is flushed with N_2 immediately before addition to cell cultures, the DNA-damaging and chromosome-breaking action is virtually abolished The active concentrations of ascorbic acid–Cu^{2+} are relatively high as compared with those of the potent carcinogen and mutagen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) which triggers a DNA repair synthesis at concentrations varying from 10^{-7} to 2×10^{-4} M (ref 5), and induces chromosome aberrations at doses of 5×10^{-6} to 6×10^{-5} M (ref 13), or 4-nitroquinoline-1-oxide (4NQO) which starts to elicit these two phenomena at 2×10^{-7} M and 4×10^{-4} M, respectively[5,14].

A significant frequency of *his*$^+$ revertants per surviving bacterial population was induced by a short (15 min) exposure to the ascorbic acid–Cu^{2+} mixture (Table 2). At equimolar concentrations freshly prepared ascorbic acid does not exert a detectable mutagenic effect. The range of the active concentrations of the ascorbic acid–Cu^{2+} mixture is relatively narrow and thus can be missed easily. For comparison, the mutagenic effect of MNNG at these relatively high concentrations has been added to Table 2. In this connection it should be noted that about 100 new *his*$^+$ revertants which require a dose of about 2×10^{-2} M ascorbic acid–Cu^{2+} are induced by as low a concentration of MNNG as 10^{-4} M Copper ions at the concentrations of 10^{-5}–10^{-6} that we used had no detectable effect on the mutation frequency of *S. typhimurium* The latter observation agrees with the lack of mutagenic activity of CuCl and $CuCl_2$ in the rec assay of *Bacillus subtilis*[15].

Considerable evidence has been accumulated to show that oxidation products of ascorbic acid are mutagenic for

Fig. 2 Unscheduled incorporation of ^{3}H-TdR into nuclei of non-proliferating cultured human fibroblasts exposed for 90 min to ascorbic acid: $CuSO_4$ mixtures (○) or an ascorbic acid solution which was bubbled with pure N_2 (Canada Liquid Air Ltd) for 1 h before application to the culture cells (●). The autoradiographic analysis of the incorporated ^{3}H-TdR and the arrest of proliferation by an arginine-deficient culture medium were described before[12]



microbial and mammalian cells: ascorbic acid in the presence of oxygen converts covalently-closed circular DNA to open circular DNA molecules (unpublished results of A. R Morgan, R. C Cone and T M. Elgert), causes single-strand breaks in DNA[6], inactivates transforming DNA of *Pneumococcus*[7], degrades RNA of bacteriophage R17 (ref. 16), induces reverse mutations in *S. typhimurium* (Table 2), fragments DNA of cultured human cells (Fig. 1) and mouse sarcoma cells[17], triggers a DNA repair synthesis (Fig. 2) and elicits chromosome breaks and exchanges (Table 1), as do most, if not all chemical mutagens The precise molecular nature of the ultimate mutagenic metabolite of ascorbic acid is unknown. Whether hydroxyl radicals that are generated in the interaction of Cu$^+$ with H_2O_2, which is in turn produced by the oxidation–reduction activity of the ascorbic acid–dehydroascorbic acid system[18], are responsible for the observed DNA fragmentation and the genetic changes is difficult to assess at present.

It is also difficult to evaluate the degree of genetic hazard posed by vitamin C, its decomposition products, and its interaction with metal ions to man. The effective dose of ascorbic acid in the presence of oxygen or cupric ions varies from 5×10^{-5} M for single-strand scissions of DNA[6] and cleavage of PM2 DNA (unpublished results of A. R. Morgan *et al.*) to between 1 and 8×10^{-4} M for the induction of DNA lesions and chromosome aberrations in cultured human fibroblasts Several potent mutagens, including MNNG, MMS and EMS require also high concentrations to trigger a DNA repair synthesis[4,5]. Thus, the active dose of ascorbic acid products is only two- to tenfold greater than that of potent mutagens if mammalian cells are considered. In the microbial test system, however, ascorbic acid–Cu^{2+} was only weakly mutagenic as compared with MNNG. A too simple application of these *in vitro* data to man can lead to erroneous conclusions. Catalyses may inhibit ascorbic acid-initiated DNA cleavage within the human body. Metabolic products of ascorbic acid may reach the intranuclear DNA molecules only if present in excessive amounts. The ascorbic acid–metal mixtures may have no effect because of the lack of free cupric or ferric ions within cells. Nevertheless, the potential mutagenic capacity of ascorbic acid products should be taken into consideration when a potential health hazard due to the addition of relatively large quantities of vitamin C to food products which contain nitrosatable compounds is evaluated.

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Received January 9; accepted February 26, 1976

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Conformational differences in bacterial ribosomal RNAs in non-denaturing conditions

THE conservative nature of bacterial ribosomal RNA (rRNA) is clear from the narrow range of its guanosine–cytosine (GC) content compared with the base composition of whole cell DNA. The majority of bacterial DNAs contain from 35 to 75% GC, whereas the GC content of rRNA is restricted to a narrow range of 50–53% (ref. 1). Indeed, the ribosomal DNA (rDNA) of many bacterial species can be isolated from sheared preparations of whole cell DNA solely on the basis of its GC content. Furthermore, nucleic acid hybridisation has indicated considerable homology between the rRNAs of diverse bacterial species[2-4] and helped provide phylogenetic support for taxonomic relationships. In an investigation of 22 different bacteria Pace and Campbell found that organisms whose rRNA showed closer homology to *Escherichia coli* rRNA, showed less rRNA homology to *Bacillus stearothermophilus* rRNA and vice versa[2]. Both sedimentation and gel electrophoresis studies in non-denaturing conditions have shown very small, if any, migratory differences between either the 23S or 16S rRNA species of different bacterial species[5,6]. By using a modified buffer system coupled with double labelling, we have found that both the large and small species of rRNA from *Streptococcus faecalis*, *B. subtilis*, and *E. coli* can be all distinguished on the basis of significant differences in their mobility in both composite polyacrylamide–Agarose gels and polyacrylamide gels.

The relevant bacterial species were labelled with ^{3}H-5-uridine or ^{14}C-2-uridine and the rRNA was extracted and electrophoresed in composite polyacrylamide–Agarose gels as described in the legend to Fig. 1.

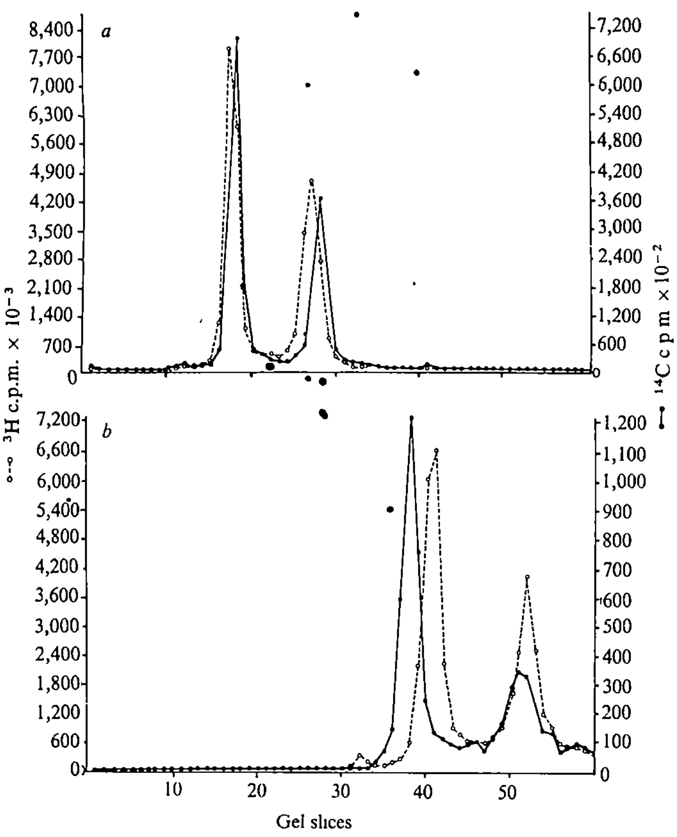
Figure 1*a* shows the clear separation of both 23S and 16S peaks of the rRNAs from *S. faecalis* and *E coli*. Similarly, good separation was achieved between *S. faecalis* and *B. subtilis* (Fig. 1*b*), and *E. coli* and *B. subtilis* (Fig. 1*c*). The 23S and 16S peaks of all species were clearly separated by one or more gel slices. It is not possible to make comparisons between different gels, since the buffer temperature was not controlled during electrophoresis and the runs were made on different days. It is important to note that these differences would not be observable if absorbance measurements were used and thus would be overlooked in electrophoretic studies using unlabelled rRNA.

These migratory differences were confirmed by electrophoresis in similar conditions in 3% polyacrylamide gels. The only exception to the migratory patterns seen in composite gels was that the 16S peaks of *E. coli* and *B. subtilis* coelectrophoresed (Fig. 2).

Electrophoresis of these rRNA species was also carried out in composite polyacrylamide–Agarose gels with a standard internal buffer concentration of 1E which is commonly used in RNA electrophoresis[6]. In these conditions, all the bacterial rRNAs coelectrophoresed with the exception of *B. subtilis* 23S rRNA, which migrated slightly behind the *S. faecalis* and *E. coli* 23S RNA peaks (Fig. 3).

If these migratory differences were to be interpreted according to the method of Loening[6], where distance migrated is inversely proportional to the logarithmic value of the molecular weight, they would be equivalent to 10% differences in molecular weight. Electrophoretic migration in non-denaturing conditions has, however, been shown to be primarily dependent on conformation based on the partially double-stranded nature of single-stranded RNA in aqueous solution. Temperature, ionic strength, *p*H, base sequence and base composition of RNA all seem to be factors which determine conformation.

A common buffer used for electrophoresis of RNA is the 1E buffer of Loening, which is at a physiological *p*H and sodium ion concentration. The *p*H and ion concentration assure that at room temperature the rRNA in gels assumes a conformation similar or identical to its native conformation. Successful



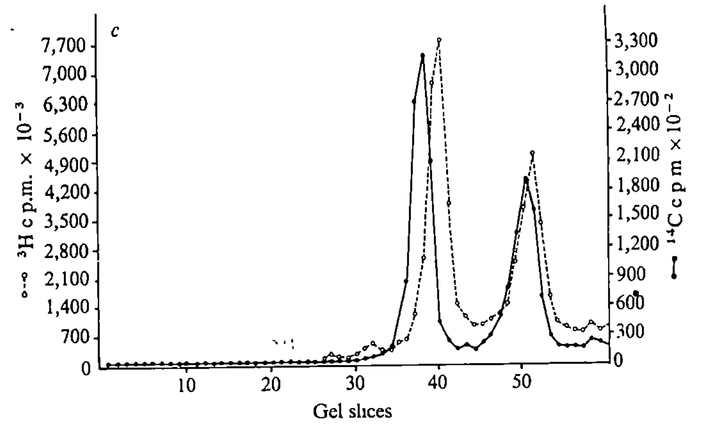


Fig. 1 Bacteria were labelled for 6–12 h of logarithmic growth with either ^{3}H-5-uridine or ^{14}C-2-uridine (Schwartz–Mann) to facilitate uniform labelling of mature rRNA. RNA was extracted with phenol at 40 °C with sodium dodecyl sulphate (SDS)[11]. All the Gram-positive bacteria were treated before extraction with 0.3% lysozyme for 15 min at 45 °C which partially digested the cell wall. RNA was precipitated from the final aqueous phase by addition of cold 0.8 M NaCl and absolute ethanol at −20 °C. After incubation overnight at −20 °C the mixture was centrifuged and the pellet resuspended in 3 M sodium acetate and 5 mM EDTA to selectively precipitate RNA and remove residual DNA. This solution was centrifuged and the pellet resuspended in a small volume of E buffer plus 0.5% SDS and stored at −70 °C (ref. 6). RNA concentration was measured by orcinol determination[12]. Generally, 5 µg of each RNA sample in 5–10 µl of E buffer plus 0.5% SDS were added to each gel. RNA samples to be analysed were mixed and placed on the same gel. Preliminary experiments had indicated that the electrophoretic profiles of rRNAs obtained by coextraction did not differ from those obtained by mixing just before the gel run. Polyacrylamide–Agarose gels were prepared by the method of Peacock and Dingman[13] with the following modifications. The buffer used in gel preparations was 3E rather than 9E so that the final buffer concentration in the gels was 1/3 E rather than 1E[13]. The gels were 2.6% acrylamide, 9–10 cm long and 0.5 cm in diameter. The procedure for gel electrophoresis was that of Loening[6]. The samples were electrophoresed for 1.5 h at 5 mA per gel. In all graphs the direction of electrophoretic migration is from left to right. Because we were interested only in the relative migration of the RNA samples, unwanted sections of the gel were removed from both the top and bottom of the gel. The gel slices referred to on the abscissa therefore do not reflect total distance migrated. After completion of the electrophoretic run the gels were fixed in 1M acetic acid for 15 min, washed with water for at least 30 min, frozen and sliced with a gel slicer (9 slices cm^{-1}). Each slice was placed in a vial with scintillation fluid (PPO–POPOP)+5% protosol tissue solubiliser, prewarmed to 37 °C, and shaken overnight at 37 °C. Vials were counted in a Packard TriCarb Liquid Scintillation Spectrometer Model 3003 *a*, *S. faecalis*, ----; *E. coli*, ——. *b*, *B. subtilis*, ----; *S. faecalis*, ——. *c*, *B. subtilis*, ----; *E. coli*, ——.

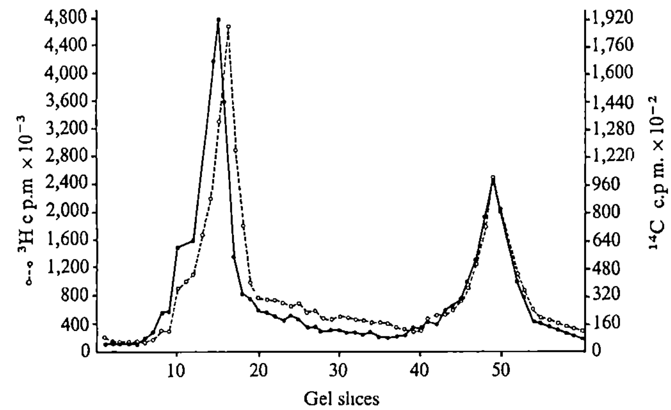
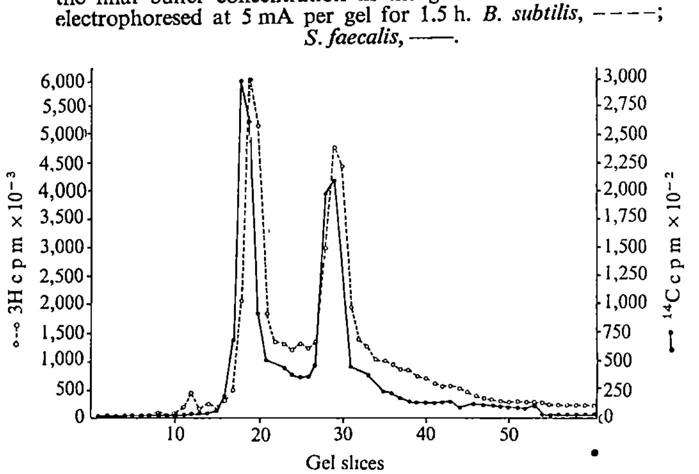


Fig. 2 3% Acrylamide gels were prepared using the monomer mix (acrylamide-*bis*-acrylamide) of the composite gels. Again, the final concentration of buffer in the gels was 1/3 E. The gels were 14 cm long and 0.6 cm in diameter. Electrophoresis was at 10 mA per gel for 2.5 h. *B. subtilis*, ----; *E. coli*, ——.

ribosomal reconstitution experiments carried out in similar buffer conditions with homologous rRNA and ribosomal proteins suggests that the rRNA is in a conformation resembling its native state[7]. Successful ribosomal reconstitutions are possible using heterologous rRNA and ribosomal proteins, indicating that the native conformations of rRNAs of different bacterial species have been conserved[7]. It is, therefore, not surprising that bacterial rRNAs coelectrophorese in 1E buffer gels.

The differences in migration of both 23S and 16S rRNA seen in Figs 1 and 2 can be attributed to the use of a 1/3E buffer within gels. The lower sodium ion concentration of this buffer may no longer be able to stabilise the secondary and tertiary interactions of segments of the rRNA, thus leading to partial denaturation. The extent of denaturation is probably determined by the GC content of the double-stranded loops and their base

Fig. 3 Composite polyacrylamide–Agarose gels were prepared as in Fig. 1, except the buffer used in gel preparation was 9E, so the final buffer concentration in the gels was 1E. Gels were electrophoresed at 5 mA per gel for 1.5 h. *B. subtilis*, ----; *S. faecalis*, ——.



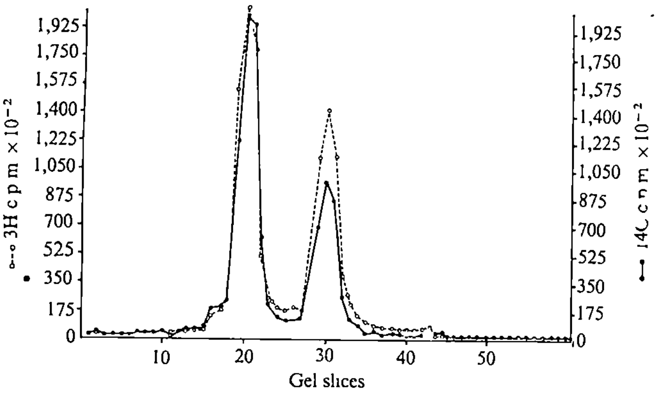


Fig. 4 Formamide gels were prepared according to the method of Staynov *et al.*[14]. All other details of the electrophoretic procedures are the same as described in Fig. 1 except the E buffer used in the electrophoresis chambers was adjusted to *p*H 9.0 rather than 7.8. *B. subtilis*, ----; *E. coli*, ——.

sequence. Therefore, rRNAs with a higher GC content presumably retain more stable double-stranded loops, pack tighter, and behave as smaller (lighter) particles during electrophoresis or sedimentation.

We believe that the electrophoretic differences we observed in the 1/3E buffer may be due to small differences in the GC content of the rRNAs of these particular bacterial species. Determinations of their respective GC contents are in progress.

Owing to the extended length of the labelling period, we do not believe any of the species of rRNA described are precursor RNAs. Morris and Schuap have separately reported multiple conformational forms of both 23S and 16S rRNAs from *E. coli*[8,9]. These forms were only seen, however, when special conditions such as a *p*H gradient[8] or electrophoresis for 7.5 h at 0 °C (ref. 9) were used. We have not seen any multiple peaks in our electrophoresis conditions.

To test our assumption that the differences we observe in non-denaturing gels were due primarily to conformational difference, we ran the rRNA samples on formamide gels. Formamide essentially destroys all secondary bonding and reduces migration to a dependency on the primary polynucleotide sequence[10]. In all cases we found coelectrophoresis of the different bacterial rRNAs. A single example is shown in Fig. 4. Thus, no molecular weight differences were seen between the respective 23S and 16S rRNA of the different bacterial species studied.

Using a combination of lowered ionic strength in our gels and double labelling of rRNAs we were able to show major migrational differences in the electrophoretic mobility of non-denatured rRNA from various bacterial species. Extension of this work could lead to differential rRNA profiles of bacteria; which might be useful in the identification of non-cultivable pathogens, or to help establish phylogenetic relationships between different bacterial species.

This work was supported by grants from the US National Institutes of Health. We thank Dr Paul Sypherd for helpful discussion.

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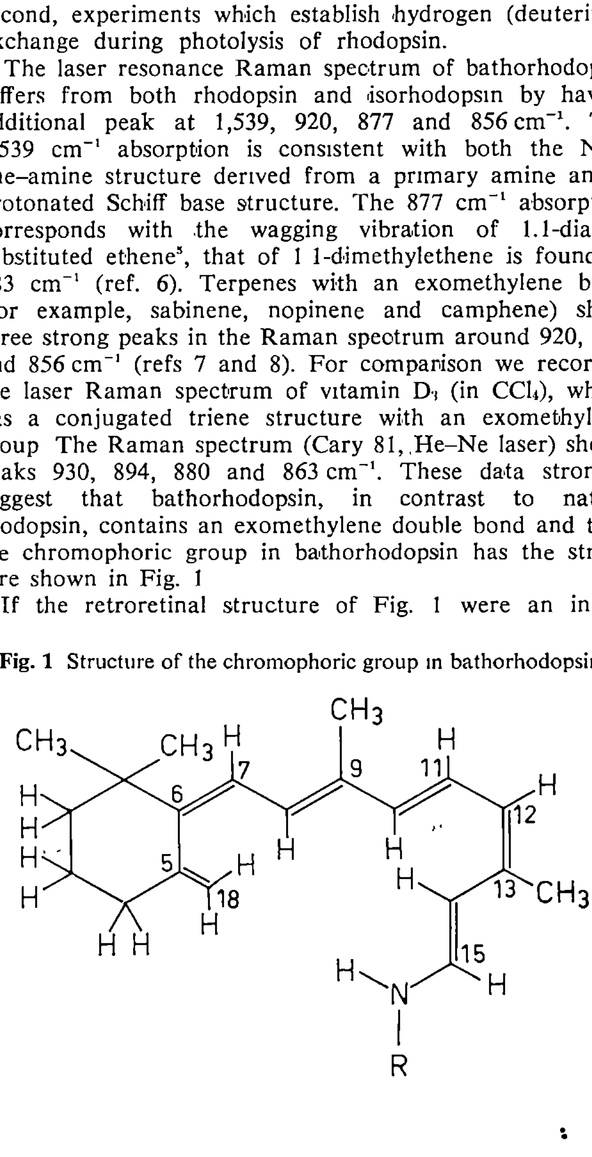
Structure of the chromophoric group in bathorhodopsin

IN the photolysis of the visual pigment rhodopsin the intermediate first observed is bathorhodopsin[1,2] (formerly called prelumirhodopsin). It is generally held that isomerisation of 11-*cis* to all-*trans* retinal occurs during this initial step. On theoretical grounds, however, an hexaene–amine structure (Fig. 1) has been proposed as an alternative for the chromophore of bathorodopsin (ref 3 and unpublished results of K. Van der Meer, J. J. C. Mulder and J.L.). This chromophore has an exomethylene double bond between the atoms C5 and C18 and the nitrogen of its ene–amine moiety derives from the ε-amino group of a lysine residue of opsin. It is a retrotautomer of the chromophoric group in native rhodopsin which is a protonated Schiff base of 11-*cis* retinal We now present two lines of evidence which support the structure depicted in Fig. 1: first, the interpretation of a recently published laser resonance Raman spectrum of bathorhodopsin[4], and second, experiments which establish hydrogen (deuterium) exchange during photolysis of rhodopsin.

The laser resonance Raman spectrum of bathorhodopsin differs from both rhodopsin and isorhodopsin by having additional peak at 1,539, 920, 877 and 856 cm^{-1}. The 1,539 cm^{-1} absorption is consistent with both the N–H ene–amine structure derived from a primary amine and a protonated Schiff base structure. The 877 cm^{-1} absorption corresponds with the wagging vibration of 1.1-dialkyl substituted ethene[5], that of 1 1-dimethylethene is found at 883 cm^{-1} (ref. 6). Terpenes with an exomethylene bond (for example, sabinene, nopinene and camphene) show three strong peaks in the Raman spectrum around 920, 877 and 856 cm^{-1} (refs 7 and 8). For comparison we recorded the laser Raman spectrum of vitamin D_3 (in CCl_4), which has a conjugated triene structure with an exomethylene group The Raman spectrum (Cary 81, He–Ne laser) shows peaks 930, 894, 880 and 863 cm^{-1}. These data strongly suggest that bathorhodopsin, in contrast to native rhodopsin, contains an exomethylene double bond and that the chromophoric group in bathorhodopsin has the structure shown in Fig. 1

If the retroretinal structure of Fig. 1 were an inter-

Fig. 1 Structure of the chromophoric group in bathorhodopsin.



mediate in the rhodopsin photolysis in the process of reconstituting the 18-CH_3 group in all-*trans* retinal, the ultimate product of rhodopsin photolysis *in vitro,* a hydrogen atom (proton) should be reattached to the 18-CH_2 group in one of the thermal reactions following the formation of bathorhodopsin. Then, hydrogen exchange between chromophore and apoprotein during photolysis might be detected by means of deuteration (or tritiation) of the latter and analysis of the chromophore. In spite of the negative outcome of an earlier attempt to detect tritium exchange during rhodopsin photolysis[3], various considerations led us to reinvestigate this approach in a slightly modified manner.

In a first attempt, rhodopsin, isolated as rod outer segments from 60 bovine eyes by a sucrose gradient technique[9], was washed repeatedly with D_2O and lyophilised. After incubation in D_2O in various conditions, the sample was irradiated with orange light for 30 min. The suspension was brought to 60% (v/v) ethanol and extracted with hexane. The retinal was isolated (thin-layer and high pressure liquid chromatography) in pure form. The samples were placed in an AEI MS-902 mass spectrometer through the direct, heatable introduction system. Spectra were taken, slowly heating the sample from 40 to 120 °C. From the retinal isolated, eight complete mass spectra, each at probe temperatures of 40, 60, 90, 100 and 120 °C, were obtained. At 100 and 120 °C, 20 spectra in the parent peak region were taken. The mass spectra thus obtained closely resemble those published for all-*trans* retinal[10]. In three independent experiments in which the incubation conditions with D_2O are varied (0 °C, 14 h; 0 °C, 18 h; 20 °C, 18 h), however, no deuterium incorporation was found in the chromophore.

This result might be explained by the possibility that D_2O has no access to the region of direct protein–chromophore interactions in rhodopsin Chances for more complete deuteration of the apoprotein could be better in opsin, where the chromophore is absent. Therefore, opsin, isolated as rod outer segments in room light[9], was washed repeatedly with D_2O and lyophilised The preparation was resuspended in D_2O and after incubation for various times a fivefold molar excess of 11-*cis* retinal was added. After 1.5 h rhodopsin regeneration, the preparation was centrifuged and the pellet was extracted three times with hexane to remove the excess of retinal. The residue containing regenerated rhodopsin was resuspended in D_2O. Samples of illuminated and unilluminated rhodopsin were extracted, purified and submitted to mass spectrometry as described above. The results are presented in Table 1.

In five independent experiments, deuterium incorporation to the extent of 6 ± 1% of one D atom was found in the all-*trans* retinal extracted from illuminated samples, as determined from the peaks of $m/e = 284$, 285 and 286, calculated according to Beynon and Williams[11]. In the control experiments, the chromophore extracted from unilluminated samples (largely 11-*cis* retinal as shown by thin-layer chromatography) did not show any deuterium incorporation. This demonstrates that deuterium is incorporated in the chromophore as a result of illumination.

The accepted mechanism for the photolysis of rhodopsin is based on a photochemical *cis-trans* isomerisation of the chromophoric group[12]. Since retinal does not show H-D exchange, in either thermal or photochemical conditions, our observation of a light-induced H-D exchange in the chromophore of rhodopsin is difficult to explain by such a model. We have proposed another mechanism, in which a hydrogen shift is introduced as the photochemical step, resulting in a hexaene-amine moiety as structure for early photointermediates of rhodopsin photolysis (unpublished results of K. Van der Meer, J. J. C Mulder and J. L) This model leaves two possibilities for the exchange of hydrogen between chromophore and apoprotein. The hydrogen donated to the apoprotein is either not that which is returned in reconstituting the chromophore or it is later reattached to the chromophore.

Table 1 Percentage of deuterium incorporation in retinal extracted from rhodopsin obtained by regeneration from D_2O-incubated opsin and 11-*cis* retinal

Opsin incubation with D_2O (°C, h)	11-*cis* Retinal from rhodopsin before illumination	all-*trans* Retinal from rhodopsin after illumination
20, 1	—	6
20, 5	—	5
20, 14	0	5
20, 18	0	7
30, 14	—	7

In the first case, 100% monodeuteration could be expected if the hydrogen-donating site on the apoprotein is completely deuterated. But our observation that the percentage of deuterium exchange is low and independent of the time during which opsin is incubated with D_2O (Table 1), suggests that such a hydrogen-donating site may not exist In the second case, one would not necessarily observe H–D exchange; exchange might occur during the time the shuttling hydrogen spends on the protein. We suggest that the very short lifetimes of the photointermediates, bearing a hexaene–amine as chromophore, are responsible for the relatively low deuterium incorporation in retinal during photolysis.

In conclusion, the laser resonance Raman spectrum of bathorhodopsin and the occurrence of hydrogen exchange between chromophore and apoprotein during photolysis support a hydrogen shift as the primary event in the photolysis of rhodopsin.

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Received August 4, 1975, accepted February 19, 1976.

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Crystal structure of cholesterol monohydrate

Cholesterol, almost water insoluble, normally occurs in the body bound to lipoprotein, or incorporated in lipid aggregations such as bile micelles, or within certain biomembranes. When cholesterol levels are abnormally high, single crystals of the monohydrate tend to deposit; in bile, clumps of these crystals form gallstones[1]. Crystals of cholesterol monohydrate also

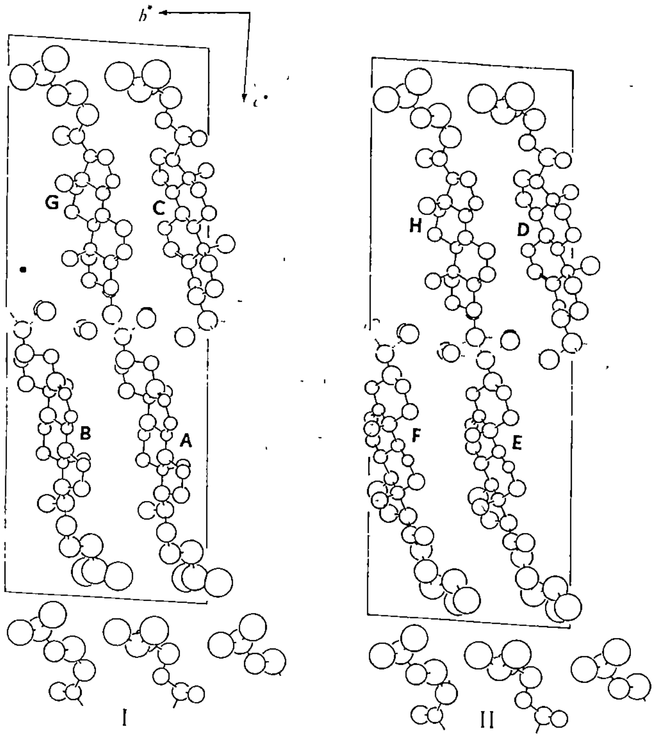


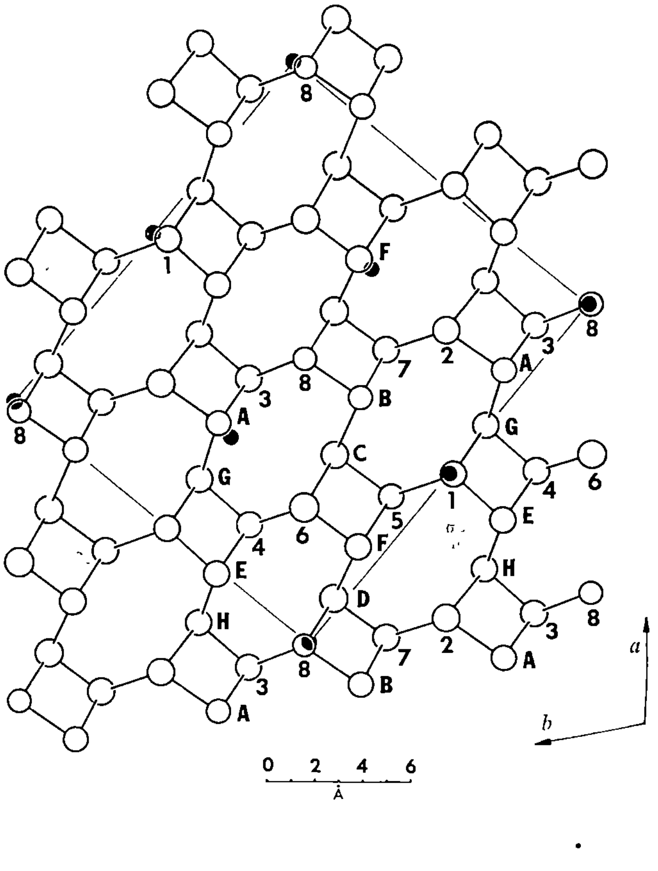
Fig. 1 Crystal structure of cholesterol monohydrate projected down *a* (into page), with II superposing on I to give the complete structure. Molecules in the pairs (A, B), (E, F) are related by translations *b*/2, and in the pairs (C, D), (G, H) by *a*/2. Circles represent isotropic thermal motion, being drawn at the level of 50% probability of enclosing an atom.

occur in atherosclerotic lesions[2]. Earlier reports of crystal data for cholesterol monohydrate are not all consistent, although they indicate a structure of unusual complexity (refs 1, 3–5 and S. H. Kim and W. Warrant, personal communication). The crystal structure (Fig. 1) has now been determined by a molecular replacement method which was developed primarily for protein crystallography[6].

A crystal of dimensions 0.4×0.15×0.10 mm along the *a*, *b*, *c* directions, grown from acetone–water solution and sealed in a lacquer coating, was examined by CuKα X-ray precession photography and by computer-controlled diffractometer. From comparison of the $h\bar{h}l$ net with Fig. 2 in ref. 1, the crystalline form appears to be the same as described previously[1,3]. The diffraction symmetry may well be mistaken for monoclinic, with $(4\bar{4}1)^*$ as the approximate twofold axis. There are, however, significant differences in intensities of rows such as 02*l* and 20*l* which reveal the true triclinic symmetry. The space group is thus P1, with reduced cell parameters $a=12.39$, $b=12.41$, $c=34.36$ Å, $\alpha=91.9$, $\beta=98.1$, $\gamma=100.8°$. The density (1.045 g cm^{-3}) measured for a crystal from the same mother liquor, and quickly transferred and suspended in a sucrose–water solution, agrees with the calculated density (1.048 g cm^{-3}) assuming the unit cell to contain $8(C_{27}H_{46}O).8H_2O$ (molecular weight, 3,240). Among the measured 10,157 independent X-ray reflections with $\sin\theta/\lambda < 0.5$ Å^{-1} ($d_{\min}=1$ Å) only 2,480 with intensity $I > 2\sigma(I)$ were obtained, and used in the structure determination. The high proportion of weak reflections was due to the large amplitudes of atomic thermal vibrations (overall isotropic $B=7.6$ Å^2) and to a remarkable systematic absence of reflections *hkl* with *h* and *k* both odd. These absences were previously assumed to indicate macroscopic twinning on (001), consistent with the almost equidimensional *a* and *b* cell edges[1]. It is, however, impossible to resolve the reciprocal lattice reported here into two identical sublattices with a common *c**, because the angles α* and β* are significantly different (86.5° and 81.4° respectively). It seemed that the twinning must be within the cell unit. A structural model, later shown to be correct, was based on two nearly identical but differently oriented subcells with translations (*a*, *b*/2) and (*b*/2, *a*). The first subcell structure (for example molecules A, E and B, F, Fig. 1) would contribute no diffracted intensity to reflections *hkl* with *k* odd, and the second (for example, molecules C, G and D, H) would contribute nothing to those with *h* odd, thus explaining the systematic absences. This model was consistent with the maps of the Patterson rotational and translational searches[7] and was helpful in their interpretation. Eight 20-atom cholesterol fragments were located by comparing the observed Patterson function with that of the same artificial 20-atom structure which was recently used in the crystal structure determination of cholesteryl myristate[8]. All 232 non-hydrogen atoms were found in subsequent electron density maps. Positional and isotropic thermal parameters were refined by a block diagonal least squares procedure to a present *R* index of 0.12. Estimated standard deviations in bond lengths are about 0.06 Å. Hydrogen atoms were included at calculated positions by assuming C–H bond distances of 1.0 Å and tetrahedral or trigonal bond angles. Hydrogen-bonded hydrogen atoms were not found and thus not included in structure factor calculations. The C(17) chain atoms have large thermal mean square amplitudes of vibration, ranging from 0.25 to 0.40 Å^2, but there is no evidence of disorder with respect to different conformers.

The structure shows a stacking of bilayers of thickness $d_{001}=33.9$ Å. The ring systems of all cholesterols are nearly parallel to (111) with the long axes nearly along $[\bar{1}24]$, which makes an angle of 17.4° with *c**. Molecules such as A and B which are related by subcell translations have the same conformation. Molecules such as A and G which are related by

Fig. 2 A hydrogen-bonded sheet, projected down *c** (into page). Open circles represent thermal parameters for oxygen atoms, as in Fig. 1. Hydroxyl groups are labelled alphabetically and water oxygen atoms numerically. Solid circles represent the hydroxyl ion configuration in the (001) plane of hydroxyapatite. Outlined is a supercell of the hexagonal hydroxyapatite structure.



approximate local twofold symmetry have similar conformations, and molecules such as A and E within the same subcell have different conformations. Thus in A, the C(17) chain is twisted towards the β side of the molecule, whereas in E the twist is towards the α side, so that the two chains are approximately parallel in the crystal. None of the molecules has a fully extended C(17) chain, such as was found for one of the two different rotamers in cholesteryl myristate. The partial folding of all C(17) chains in cholesterol monohydrate results in a condensed structure at the centre of the bilayer.

In projection down [$\bar{1}$24], the subcell for cholesterol packing gives a net with $a_s=12.19$, $b_s=6.10$ Å, and $\gamma_s=103.4°$ and an area of 36.2 Å^2 per molecule. In the bilayers of the crystal structure of cholesteryl myristate[8], the subcell for cholesterol packing also contains two parallel molecules, the cross-sectional area being 36.6 Å^2 per molecule. The packing, however, is different. In cholesterol monohydrate, the projecting C(18) and C(19) methyl groups from discrete pairs of molecules (for example A and F) are at van der Waal's distances, whereas in cholesteryl myristate, such distances occur between a given molecule and two neighbouring molecules, thereby forming infinite chains of methyl–methyl interactions.

The C(3) hydroxyl groups and water molecules are hydrogen bonded to form a pleated sheet at the bilayer interface (Fig. 2.) Each oxygen atom forms three hydrogen bonds, so that there are 24 distinct O· · ·O distances which range from 2.68 to 3.01 Å. The average distance (2.86 Å) is on the long side of the reported distribution for other organic hydrates (average 2.80 Å with an e.s.d. of 0.09 Å (ref. 11)). This, together with the rather high values for the mean square thermal amplitudes of vibration of the oxygen atoms ($0.11 < \overline{u^2} < 0.16$ Å^2), suggests that the hydrogen-bonding network is weak, and that the water molecules have an important space-filling role in stabilising the structure during crystal growth. Migration of water molecules along the tunnels parallel to a, in which they lie (Fig. 1), provides an explanation for the ease with which the crystals dehydrate on exposure to air.

Epitaxial relationships between crystal structures may be important in nucleating the deposit of crystals in biological systems[10]. Crystals of both hydroxyapatite and cholesterol monohydrate usually develop (001) faces. The hydroxyl ions of hydroxyapatite are in planes parallel to (001) with O–H bonds favourably oriented for hydrogen bonding at the (001) crystal surface[11]. A fit of the (001) planes shows close superposition of hydrogen bonding groups from the two crystal structures. The corresponding superlattice nets (Fig. 2) have $a=18.84$, $b=16.31$ Å, $\gamma=90°$ for hydroxyapatite and $a=19.13$, $b=15.84$ Å, and $\gamma=90.1°$ for cholesterol monohydrate. With the intervention of a transition layer of hydrogen-bonded water molecules, a microcrystal of either structure may serve as a nucleus for growth of the other. Since atherosclerotic plaque from recently deceased patients has been shown to contain crystalline hydroxyapatite[12], it is possible that cholesterol monohydrate is implicated in the calcification often associated with later stages in the development of such lesions.

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Accessible area, packing volumes and interaction surfaces of globular proteins

I RE-EXAMINE here the recently published empirical equations of Chothia[1] for the accessible surface area of 12 monomeric proteins, and demonstrate that accessible surface area of monomeric proteins varies as the 2/3 power of the molecular weight. The total packing volumes of proteins are obtained from the sum of the individual residues, and the approximation of proteins as spheres is used to predict the loss of accessible surface on association of two proteins. The treatment is extended to calculate the proportion of the monomer surface buried in the formation of higher oligomers.

The total accessible area (in Å^2) of the polypeptide chain in the extended form, A_t, was found to be a linear function of molecular weight, M, as it should be since each additional residue contributes on average a fixed area. If A_s is defined as the accessible surface area of the folded protein chain, $A_b=A_t-A_s$ is the accessible surface area that is buried when the protein folds. Chothia proposed that

$$A_t=1.44\,M \tag{1}$$

$$A_b=0.859\,M+0.774\times10^{-5}\,M^2 \tag{2}$$

It follows that

$$A_s=0.581\,M-0.774\times10^{-5}\,M^2 \tag{3}$$

Equation 3 implies that the accessible surface is zero at $M=75{,}000$, which does not seem reasonable. Moreover, since the proteins are globular it is reasonable to assume that A_s should should vary as $M^{2/3}$.

Using Chothia's data for 12 monomeric proteins, I found by least squares analysis that

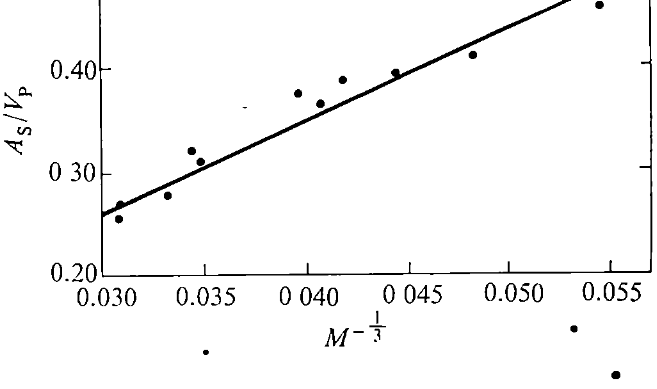
$$A_t=(1.449\pm0.006)\,M\,(\text{standard error}=417\text{ Å}^2) \tag{4}$$

$$A_s=(11.116\pm0.161)\,M^{2/3}\,(\text{standard error}=401\text{ Å}^2) \tag{5}$$

The standard error of the fit to the data in equation 5 is not much below the standard error of 428 Å^2 obtained from the data using equation (3); there are several other reasons, however, that demonstrate that equation 5 has the correct form. First, least squares analysis of log A_s as a function of log M yielded 0.685 ± 0.027 as the slope, verifying the 2/3 exponent for M in equation 5. A second argument for equation 5 comes from the area buried on folding the protein. Subtraction of equation 5 from equation 4 gives

$$A_b=1.449\,M-11.116\,M^{2/3} \tag{6}$$

Fig. 1 Molecular weight dependence of the surface–volume ratio. The solid line corresponds to equation 8. A_s is the accessible surface area and V_p is the total packing volume.



This equation yields zero buried area at $M=451$, a reasonable physical limit. Outside the range of M between 10^4 and 3.5×10^4 equations (2) and (6) differ significantly.

I have estimated the total packing volume of these proteins by simply summing the packing volumes of all protein residues in the primary sequences using the volumes of buried residues given by Chothia[1]. The value for the arginyl residue was estimated from the correlation between residue partial specific volumes[2] and packing volumes for the remaining 19 residues. The values calculated for lysozyme and ribonuclease agree closely with those of Richards[3]. Defining V_p as the total packing volume, I found the following empirical relationship for the 12 proteins

$$V_p=(1.273\pm0.006)\,M\,(\text{standard error}=401\ \text{Å}^3) \qquad (7)$$

From equation (5) and equation (7), the surface–volume ratio should vary as $8.733\,M^{-1/3}$. The least squares equation found is,

$$A_s/V_p=(8.768\pm0.102)\,M^{-1/3}\,(\text{standard error}=0.015\ \text{Å}^{-1}) \qquad (8)$$

Figure 1 shows this surface/volume relation to be reasonable. The exponent of M in equation 8 was verified by analysis of log A_s/V_p against log M. The exponent found was -0.331 ± 0.021.

Variation of accessible surface area as $M^{2/3}$ for these monomeric globular proteins implies that in protein–protein complexes, protein subunits may be treated as spheres. In packing volume, monomeric proteins behave as if they possessed the radius,

$$R_p=\left(\frac{3V_p}{4\pi}\right)^{1/3}=\left(\frac{3\times1.273}{4\pi}\right)^{1/3}M^{1/3}=0.6723\,M^{1/3} \qquad (9)$$

In accessible surface area, the same proteins seem to have radius R_s

$$R_s=\left(\frac{A_s}{4\pi}\right)^{1/2}=\left(\frac{11.116}{4\pi}\right)^{1/2}M^{1/3}=0.9405\,M^{1/3} \qquad (10)$$

Thus, for monomeric proteins R_s is 1.4 R_p. The physical interpretation of this is that the larger value of R_s comes from the roughness of the protein surface.

Chothia and Janin[4] have demonstrated that the packing volume in the interface between associated proteins is the same as in the proteins themselves. Therefore, on tight protein association, total packing volumes should be additive. On the other hand, some accessible surface becomes buried. Figure 2 shows how this can happen: the total packing volume is conserved in the complex, but the spheres for the surface lose area upon contact. To calculate the accessible surface area buried on association, one need only determine the surface area of overlap. Table 1 compares the results of calculations made in this way with those of Chothia and Janin[4]. While not exact, the agreement is remarkably good considering the simplicity of the model. For the dimerisation of identical monomers, the problem can be generally written as, $S_b=2\pi R_s(R_s-R_p)=0.143A_s$. S_b is the surface buried per monomer and A_s is the accessible surface of a monomer as in equation 5. Thus, in general, close association of monomers to form a stable dimer involves a loss of 14.3% of accessible surface area per monomer.

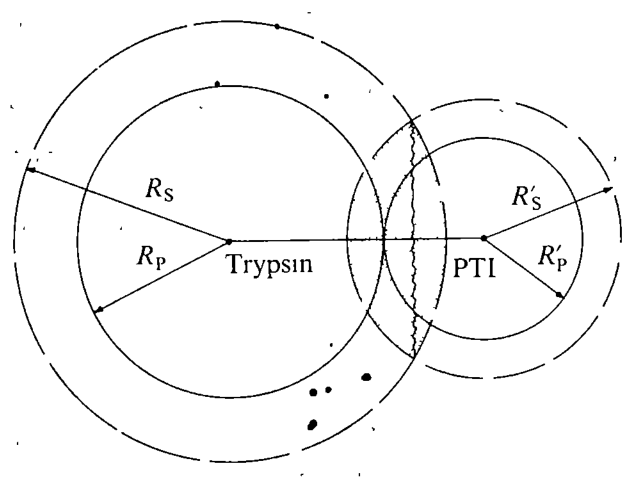


Fig. 2 Schematic diagram of association of trypsin and pancreatic trypsin inhibitor (PTI). The packing radii are represented by R_p and R_p' and the solid circles. The accessible surface radii are denoted by R_s and R_s' and the dashed circles. Accessible surface area which is buried on association is denoted by the overlap of the dashed circles (stippled area). The wavy line denotes the partition of buried accessible surface to the interacting proteins.

In a square planar tetramer of identical monomers, each monomer contacts two subunits. Thus, each monomer is predicted to lose 28.6% of its accessible surface in the tetramer. The packing of 4 spherical volume elements into a tetrahedral tetramer by this model gives a double burial of some accessible surface area. Neglecting this area of overlap, the geometrical prediction from the model is that 43% of accessible area of the monomeric subunit becomes buried on association with this type of tetramer.

The predicted value of accessible surface area for the concanavalin A monomer from equation 5 is ~ 6% lower than observed[1]. Thus, at present, it is not certain that equation 5 can be used to predict accurately accessible surface of monomers with complex arrangements of subunits in the oligomer.

Table 1 Predicted and observed changes in accessible surface area

Complex	Molecular weight	Total accessible surface area (Å²) Predicted	Total accessible surface area (Å²) Observed	Area buried in complex Value Predicted	Area buried in complex Value Observed	Area buried in complex % Predicted	Area buried in complex % Observed
Insulin dimer							
Monomer M_1	5,787	3,583	3,400	512	530	14	16
Monomer M_2	5,787	3,583	3,310	512	600	14	18
Total change				1,024	1,130		
Trypsin-PTI							
Trypsin	23,302	9,069	8,920	737	640	8	7
PTI	6,158	3,735	3,700	889	815(750)*	24	22(20)*
Total change				1,626	1,455		
Horse haemoglobin							
α chain	15,114	6,795	6,940	991	930	15	13
β chain	15,979	7,052	7,130	983	790	14	11
Total change				1,974	1,720		

*750 Å² was determined from the complex; 815 Å² was found when free PTI coordinates were used.

Nonetheless, the previous considerations indicate that a vast amount of accessible surface becomes buried during the acquisition of quaternary structure. The biological significance of this loss of surface area in relation to oligomeric protein size and structure remains to be established.

I wish to thank Christoph de Haen for discussions, and the National Institutes of Health for support.

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Received November 13, 1975; accepted March 3, 1976.

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Increasing effects of repetitive cocaine administration in the rat

Reports of chronic cocaine (and related psychomotor stimulant) administration suggest that tolerance develops to many drug effects[1–3]. A few studies of high dose cocaine administration have suggested that, on the contrary, repetitive administration may be associated with increasing effects on cocaine-induced convulsions in the rat[4,5] and monkey[6] and increasing bizarre visual and inhibitory behaviour, as well as dyskinesias, in the monkey[6]. We now report for the first time that repetitive cocaine administration can be associated with progressively increasing effects on horizontal and vertical hyperactivity as well as stereotypy in the rat.

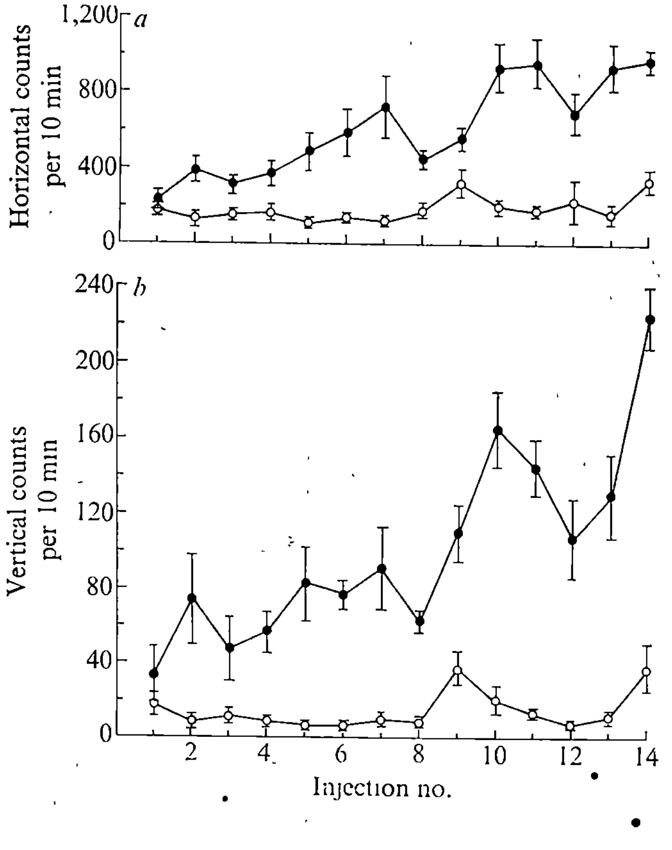
Sixteen male Sprague–Dawley rats weighing 150–185 g were maintained in groups of 3 or 4 animals per cage on a 12-h light–dark cycle. Eight rats received intraperitoneal injections of cocaine 10 mg kg^{-1} once daily, with two drug-free days each week. Eight saline-control animals received an equal volume of injection on a similar basis. Animals were removed from their home cages and tested in a 24×24×15 cm Plexiglas chamber. After a 20-min period of adaptation, animals were injected with cocaine or saline, replaced, and monitored in 10-min intervals for 90 min. Each animal was tested at the same time of day, either 0900, 1100, 1300 or 1500, during the light phase of the cycle. Cages were placed on top of 40 infrared photoelectric cells (Motron motility meter) for measurement of activity counts. Slight horizontal movements of the rats, and not just gross locomotor activity, registered as activity counts because of the density of the photocells. Five photoelectric cells positioned 13 cm above the floor of the cage measured vertical activity. Animals were also rated for degree of stereotypy ranging from 0 for absent to +4 for intense, continuous stereotypic patterns during a given 10-min interval.

The animals showed increased horizontal and vertical hyperactivity and stereotypy in response to the repeated cocaine injections. Figure 1 illustrates the increase in horizontal and vertical hyperactivity over the course of 14 injections (both $P<0.001$, 2-way analysis of variance for repeated measures). Figure 2 documents the course of the progressive development of vertical and horizontal hyperactivity, and shows an increase in both peak effects and duration. Following the first few injections, the animals' activity had returned towards the baseline by 60–90 min; while after 15 injections, the activity was still near peak levels at this time. Stereotypy ratings increased from 0.4 ± 0.1 following the first injection to 3.2 ± 0.1 following injection 14. Initially, animals displayed mild repetitive sequences moving from corner to corner, rearing on haunches, and quick circular movements of the head. These patterns were initiated earlier and became progressively more intense, redundant, and prolonged following later injections. For example, rats would engage in frantic, persistent head circling or bobbing while lying prone, or repetitively raise and lower themselves on their haunches with no change of pattern for as long as 15 min.

Conditioning phenomena, as suggested by Tilson and Reich[7], do not appear to account adequately for these progressive increases in hyperactivity and stereotypy. For injection 25, instead of receiving their usual cocaine injection, experimental animals received saline. As illustrated in Fig. 2, these animals reacted with minimal hyperactivity and stereotypy, not significantly different from saline control animals. Similar results and arguments have been presented by Segal and Mandell[8] for the progressive effects of continued amphetamine administration on behaviour, again supporting the idea that conditioning effects are not an adequate explanation of the phenomenon. Although increased blood or brain cocaine levels with chronic administration must be carefully ruled out, preliminary evidence in the rat[9] and monkey (R. Hawks and R. M. P., unpublished) suggests that the peak levels and half life of cocaine in blood and CSF do not increase with chronic administration. After 24 injections, the weights of cocaine treated animals (240 ± 11 g) were similar to that in saline controls (245 ± 9 g), indicating that differential weight loss or debilitation did not account for the progressive effects.

Our data thus suggest that repetitive administration of cocaine is capable of producing increasing hyperactivity and stereotypy in some conditions of administration. Whether tolerance can be manifested may depend not only

Fig. 1 Increasing effect of repeated cocaine injections on (*a*) horizontal activity and (*b*) vertical activity. ●, cocaine (10 mg kg^{-1}; $n=8$); ○, saline ($n=8$).



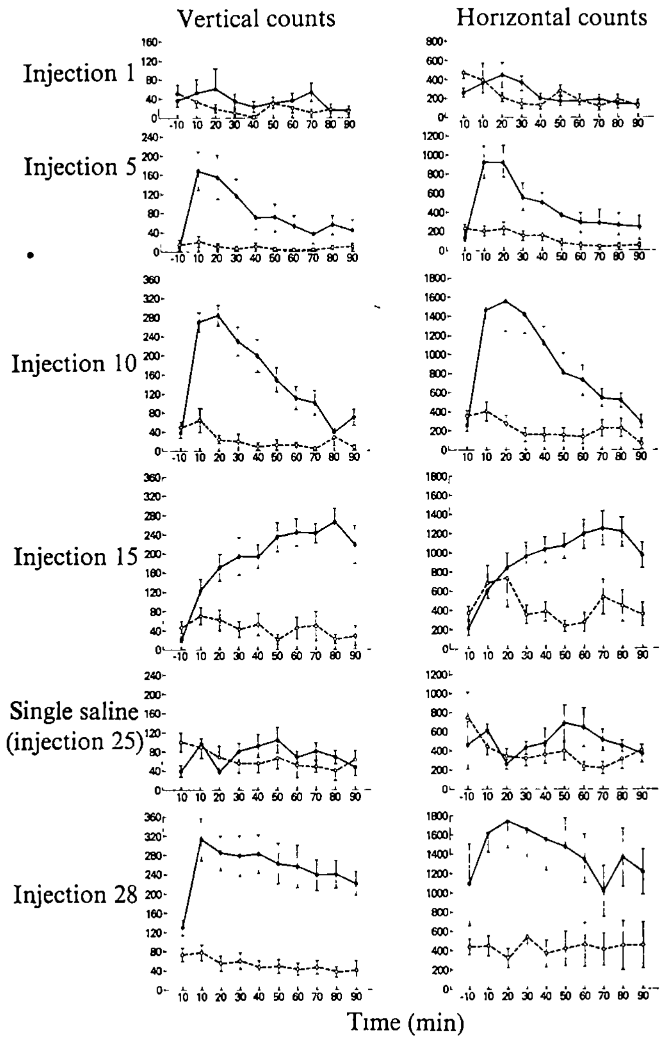


Fig. 2 Effects of repetitive injections on time course of cocaine-induced hyperactivity.

on the specific parameters studied[1-3], but also on the intermittent rather than continuous drug administration. Previous studies have documented that repetitive, intermittent, high dose cocaine administration could be associated with progressive increases in a variety of effects in the rat, dog, and monkey, including seizures, catalepsy, motor inhibition, visual tracking behaviour, and dyskinesias[4,6,10,11] That the lower doses of cocaine administration used here are also capable of producing progressive effects suggests that the phenomenon is not merely a toxic manifestation of high dose administration.

We have suggested that some progressive effects of repetitive administration of cocaine[6,12] as well as the local anaesthetic, lidocaine[13], seem very similar to those of electrical kindling[14-17] in which repetitive electrical stimulation of the limbic system is associated with increasing afterdischarge activity and eventually in major motor convulsions at previously subthreshold stimulation. Since cocaine and lidocaine produce marked effects on limbic spindles, after-discharges, and seizure activity[18,19], it is possible that a kindling-like mechanism, increasing duration and spread of electrical activity, could account for the progressive effects of lower doses of cocaine where convulsions are not involved as an endpoint. Consistent with a kindling mechanism, Ellinwood *et al.*[20] and Stripling and Ellinwood[21] have reported the increased spread of electrical discharges in cats and rats with chronic cocaine administration.

Many different biochemical mechanisms could also be involved in the apparent sensitisation to cocaine effects including pre-[22,23] or post-synaptic[24,25] alterations, or progressive changes in the activation[26,27] or binding[28] of different kinds of receptors. Rebec and Groves[29] reported that repetitive amphetamine pretreatment increased the effect of iontophoretically administered amphetamine and apomorphine on striatal and reticular formation neurones, supporting the concept of an increased receptor sensitivity with chronic stimulant administration[25]. Gunne and Jonsson[30] reported that cocaine injections (100 mg kg^{-1}) once daily for 14 d produced increasing effects on urinary, but not brain, adrenaline and noradrenaline. Study of the biochemical substrates for the progressive behavioural effects of cocaine[31] and their possible relationship to a kindling process[32], may help elucidate the mechanisms involved in the stimulant induced psychoses of man.

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Received December 15, 1975, accepted March 2, 1976.

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reviews

Fascination for discovery

Dorothy C. Hodgkin

Photo: Godfrey Argent

The Development of X-Ray Analysis. *By the late Sir Lawrence Bragg. Edited by D. C. Phillips and H. Lipson. Pp. viii + 270. (Bell: London, 1975.) £6.50.*

THIS book is a joy to read. Over and over again one can almost hear W. L. Bragg talking, as he used to talk when he was with us, always himself fascinated by the problem of solving the next crystal structure, telling us stories meanwhile about the past, mildly debunking, still illuminating.

His point of departure in this book is the discovery of X rays by Röntgen in 1895 and Röntgen's own extensive experiments on their nature. These lead to a general discussion of the evidence for the nature of light and of fundamental particles, and so to the discovery of X-ray diffraction itself, and to the experiments which followed on the structure of crystals and of atoms. In small asides he recaptures moments in the past. "I remember well a fellow student at Jeans' lectures on quantum mechanics, who used to take me aside after the lecture and give me long dissertations on just where Jeans was wrong. This was Bohr—I little knew at the time how great a man was talking to me."

It was one of the important characteristics of W. L. Bragg's own work that he believed in exact and beautiful experiments. So details are given throughout the book of the actual apparatus used and also of the conditions in the different laboratories in which research was organised. He wrote of the Cavendish laboratory of his time "When I achieved the first X-ray reflections I worked the Rumkorff coil too hard in my excitement and burnt out the platinum contact. Lincoln, the mechanic, was very annoyed, as a contact cost ten shillings, and refused to provide me with another one for a month . . . I could never have exploited my ideas about X-ray diffraction under such conditions." He moved, in fact, to Leeds, to his father's laboratory, where the facilities were on a very different scale, the consequences of W. H. Bragg's own past experience of setting up a physics laboratory of his own in Adelaide.

To solve the first, quite unknown, crystal structures, W. L. Bragg developed direct and simple arguments, leading to unambiguous answers, and based on a thorough understanding, which he illustrates fully, of optical principles and Fourier analysis. The details of structure determination changed a little as each new family of crystal structures was explored—complex ionic compounds, organic compounds, metallic structures, macromolecules. He took great pleasure in the actual structures found, and this is described in a series of chapters, which end with one on imperfect crystallisations on problems for the future. He also took pleasure in using some of the same simple arguments with which he had solved his first structures to guide the analysis of the most complicated ones, particularly of proteins. His one major omission in the book—any treatment of the statistical approach to phase determination now found in use to be very powerful—probably stems from his liking for unambiguous choices. To examine a series of very probable answers did not appeal to him, even if modern computing and his own contributions to structures solved made choice between them easy.

Not surprisingly in a book published posthumously, a few small errors appear, some of which seem sufficiently interesting historically to mention. The first is perhaps obvious—the photograph interpreted by von Laue on p17 is, as mentioned in the text, zinc blende, not copper sulphate (not interpreted till long afterwards). On p107, the map shown is not of a part of vitamin B_{12} but of the first synthetic corrin derivative—nice indeed that the electron density here was computed to demonstrate that the correct chemical synthesis had been achieved. On p112, the structure shown in Fig. 4a has a negative peak at the hexagon centre, not a positive peak as described in the text. It illustrates a deduction of Dorothy Wrinch's that the centre of the insulin unit cell should be relatively empty to account, in terms of her theory, for the observed Patterson peak heights. And finally, on p181, Bernal's first indications of the sterol structure were obtained in 1932, not 1943.

This is a book for many different kinds of reader—beginners in X-ray analysis who want to get a feel of the subject, old hands who wish to remember the past, students in other fields who would like to know something of a fascinating region of knowledge and of those who discovered it. We are deeply indebted to Professor Phillips and Professor Lipson for preparing it for publication. □

Photo: Godfrey Argent

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Vital statistics

Collected Papers of R. A. Fisher. Five Volumes. Edited by J. H. Bennett. (University of Adelaide: Adelaide, 1971–74.) A$87.00.

THE University of Adelaide has completed its five-volume edition of the scientific papers of the late Sir Ronald Fisher. These preserve the original typography of the nearly 300 items, and make available, in very agreeable form, all Fisher's important publications in scientific journals and some short articles of more general interest. Unlike other great scientists of this century, Fisher has not been widely known to the public, yet few men have so much changed the face of science. In statistical theory, he has profoundly influenced even those who differed from him philosophically. Almost every field of quantitative experimental and observational science has been affected by considerations of experimental design, of randomisation, and of the practice of statistical tests and inference, most of which can be traced to the impact of Fisher's papers and books between 1920 and 1935. To those who knew Fisher, there is special delight in again savouring the intellectual excitement that the early papers produced and the highly individual style of the author. The argument is often difficult, sometimes obscure, but always strikingly presented. To look again at "Two new properties of mathematical likelihood" is to gain insight into the real nature of statistical inference, so often concealed by the wrappings of rigour in later developments. A series of papers on the rhesus blood factor shows how statistical thought contributed to the unravelling of a genetic complexity—in retrospect simply, but highly controversial at the time. "The logic of inductive inference" remains not only a classic in the development of statistical inference but a superb example of scientific disputation enlivened by human passion. Any library with pretensions to coverage of statistics and statistical genetics needs these volumes, not merely for their historic interest but also for their continuing powers to stimulate new thought, and for the contrast with the pedestrian style and content of so much modern statistical writing.

D. J. Finney

Control theory

Control Theory in Biology and Experimental Psychology. By F. M. Toates. Pp. 264. (Hutchinson Educational: London, 1975.) £6.50.

ALTHOUGH many biologists, physiologists and psychologists realise that the concepts of control theory provide powerful unifying principles which relate not only the functional behaviour of diverse systems in their own fields, but also the physical systems of engineers and others, there is still great reserve concerning the benefits to be gained from a deep study of the theory. Although a certain amount of reluctance is due to unfamiliarity with the theoretical and numerical methods on which control theory is based, there are even more profound doubts concerning the ultimate usefulness of the subject. Before a biologist embarks on a long and often difficult study, in which he must become familiar with much of the usage of system and control engineers, he must be convinced that the outcome will offer at least one of several possible advantages. He may wish to formally pose problems which are ill-defined in non-numerical terms. Any theoretical or computer studies of a problem must produce results which at least should give reliable guides to the cause–effect relationship in his systems. He should feel confident to extend the use of any mathematical models developed to regimes beyond the scope of his experimental techniques. Above all, control theory should enable him to sharpen his choice of experiments and to plan a unique and unambiguous route of experimental deduction, for it is in the laboratory that the biologist makes his significant contributions.

There is a wealth of literature on control theory written for the specialist, but much of it is little considered outside the realms of the University. Faced with this, the biological scientist first has a considerable threshold of theoretical technique to overcome and then he must be able to discern which of the abundance of control theoretical techniques are useful to him. There is a distinct shortage of books which are written specifically for the biological sciences and which give a sufficient introduction to the mathematical rigour which will be needed. I have seen texts in which an introduction to control concepts has been attempted deliberately avoiding mathematics but, as Dr Toates knows, this is a sterile approach.

The present book approaches the problem squarely, attempting to show the importance of differential equations, and theoretical and numerical techniques for solving them, in studying the transient behaviour of any system. These methods are firmly set in a background of biological and psychological processes which, if somewhat basic, are useful enough to demonstrate the power of the techniques in analysis and deduction. The author has clearly concentrated on systems with which he is most familiar and has not tried to cover an extreme range of examples. The result is a neat text which the control engineer would find fascinating reading and which should lead the biologist gently into a more exacting study of this subject. Only when this step has been made will the considerable potential of control theory for the life sciences be realised.

J. M. Nightingale

Space odyssey

Pioneer Odyssey: Encounter with a Giant. By Richard O. Fimmel, William Swindell and Eric Burgess. Pp. 170 (NASA: Washington, 1975.) $5.50.

THIS is another in the series of commendable little books resulting from the recent (and continuing) exploration of the Solar System by NASA spacecraft. Although the book is in parts self-congratulatory, and reads in places like a publicity handout (which, in a sense, it is), the amount of worthwhile information and the spread of Jupiter pictures from Pioneer provide excellent value for the very modest cost. *Pioneer Odyssey* is highly suitable for use in introductory astronomy courses, but would serve equally well as an introduction to modern planetary science for someone specialising in another discipline but with a peripheral interest in the subject.

A lengthy introductory chapter describes Jupiter, "Giant of the Solar System"—the history of observations of the planet, how its structure probably differs from the terrestrial planets—and gives a thumbnail sketch of ideas concerning the formation of the Solar System. Although the central emphasis of the book is on the technology and techniques of this deep space mission, the section on scientific results from the Jupiter observations (and from study of the interplanetary medium) provides an excellently clear non-technical summary of the Pioneer 10 discoveries. This, and the whole book, is flawed only by the knowledge that Pioneer 11 has since made further investigations which fill out the overall picture rather more fully. I hope that the NASA/Ames team will be persuaded to produce a second edition with a fuller account of the new understanding of Jupiter in the light of Pioneer 11 data.

John Gribbin

Laser light

Plasmas and Laser Light. By T. P. Hughes. Pp. xxi+518. (Adam Hilger: London, October 1975.) £35.

LASER-related topics now hit the scientific headlines so frequently that it can become quite difficult to distinguish between actual achievement and future plans or aspirations. Confusion of this sort is perhaps inevitable in a field which is interdisciplinary, and which is still growing very rapidly. For example, in the US the relevant ERDA operating budget (supporting research and development administered by the Energy Research and Development Administration), currently totals some $90 million per annum. Research into laser isotope-separation techniques now constitutes a significant fraction of this budget, but laser fusion still continues to attract the lion's share of the funding. Within the UK a laser facility for fundamental scientific investigations has recently been established at the SRC's Rutherford Laboratory. In view of all the above developments, the recent publication of Dr Hughes' book is particularly opportune.

The historical background, some potential advantages, possible difficulties and competition for the laser-compression approach to controlled thermonuclear fusion have been outlined by me on previous occasions (*Nature*, **239**, 129 (1972); **251**, 99 and **252**, 631 (1974)). These topics constitute the dominant theme of the book, although magnetic containment and plasma diagnostics are also discussed briefly. *Plasmas and Laser Light* is a broad canvas and Dr Hughes has appropriately included an extensive treatment of ionisation and breakdown in gases and solids.

Some other sections of the book provide background information which is readily available elsewhere. In particular, much of the chapters dealing with small amplitude plasma waves, plasma spectroscopy, incoherent scattering and thermonuclear reactions have been the subject of previous specialist monographs. The main interest of this book, however, is its systematic treatment of the interaction of high intensity, coherent, light with solids, gases and plasmas, and its unifying, pedagogical treatment should therefore be of considerable value to students and younger research workers in the field. The discussion of published experimental work is very well illustrated with line diagrams, but is exhaustively comprehensive; thus the reference list is over 50 pages long. Errors in the text are few and trivial (for example, equation 2.87), and the cross referencing is excellent. It is incorrect to suggest (p54) that laser fusion has introduced a completely new regime of plasma physics; for example, parametric interactions are well known from previous controlled theromonuclear reaction (CTR) work. It is also a pity that there was no discussion of impurity-resonance scattering, nor any pictorial illustration of the growth of hydrodynamic instabilities which have been predicted in two-dimensional laser-compression computer calculations, since these may both have important consequences. Indeed, the degree of spatial symmetry required of alternative laser-compression schemes could well determine the practical feasibility of these approaches to fusion (see Lawrence Livermore Report UCRL-50021-74, p355). With these caveats, the book seems well-balanced, up-to-date, and a pleasure to read. It will certainly become a well-used text in departmental libraries, although a cheaper paperback edition might more realistically suit the pockets of many research students.

I. J. Spalding

Excited states

Excited States in Organic Chemistry. By J. A. Barltrop and J. D. Coyle. Pp. xii+376. (Wiley: London and New York, 1975.) £15.

BEFORE 1965 there were few textbooks in photochemistry but, commencing with the books by Calvert and Pitts and by Turro, the situation has been transformed. Many books are now available and both beginner and expert are well catered for. As a consequence it is necessary for any new book to offer advantages in clarity of presentation, a wide coverage coupled with modernity, appeal to particular groups of chemists, and, not least, a reasonable price, before such a book can be assured of success.

This book is written in a clear, concise style, is adequately cross referenced and develops its material in a systematic fashion. Following an introductory chapter the basic theoretical foundations of the subject are expounded in three chapters (time-independent properties, time-dependent properties and quenching of excited states). The methods specific to photochemistry are then dealt with in detail although the beginner would need to read other books to get a complete presentation of available techniques. A condensed treatment of orbital symmetry and photochemistry follows. The concluding five chapters deal with different chromophores—C=O, C=C, aromatic, nitrogen and saturated—the first three of which present the subject in considerable detail.

Both beginner and expert will profit from reading this authoritative work. The good undergraduate interested in photochemistry would grasp the scope of the subject although he would be more likely to purchase and use one of the smaller and cheaper books which give less detail and a more general coverage. The research student in photochemistry would find the book of considerable use and obtain a perspective for approaching the voluminous literature appearing annually in the Specialist Periodical Reports on Photochemistry. Experienced photochemists including inorganic and physical chemists would benefit from studying this book. It is not as encyclopaedic as Calvert and Pitts but this disadvantage is offset by being up-to-date, at least half of the basic material referenced having appeared since 1966; and in the later chapters dealing with the functional groups three-quarters of the material is new. The misprints are few and almost entirely trivial, with the exception of page 163, where electrocyclic is misprinted as electrolytic. The price is prohibitive for a student, and unfortunately, will limit sales amongst the more specialist audience who will benefit most from reading this book.

B. G. Gowenlock

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Timely review

Point Defects in Solids. Volume 2: *Semiconductors and Molecular Crystals.* Edited by James H. Crawford, Jr, and Lawrence M. Slifkin. Pp. xvi+480. (Plenum: New York and London, 1975.) $45.

THE ever-increasing number of published papers on defects in semiconductors is now vast and so it is invaluable to have timely reviews available for the purpose of coordination and also to act as starting points for newcomers to the field.

The present volume is in reality a collection of six such review articles; these may be subdivided into two groups. The last chapter by Chadwick and Sherwood is concerned primarily with diffusion processes in molecular crystals, a topic which is obviously still in its infancy. In the penultimate chapter by Walton, the effect of defects on macroscopic properties, such as thermal conductivity of various materials is discussed: this is a specialised topic and in this sense is distinct from the earlier chapters.

The first four chapters deal primarily with the effects of radiation on crystals and contain discussions of defect creation, the structure of defects and their effects on the optical and electrical properties of the materials; there is also a chapter on impurity and self-diffusion which are of course not unrelated topics. The styles of these articles vary enormously and their contents also reflect the interests and points of view of the authors. The first by Corbett and Bourgoin is a mixture of sensible speculation, which should provoke further work, and a very comprehensive cataloguing of data, with over 400 references cited. By contrast, chapter four by Watkins on electron spin resonance is completely conservative in its approach, which is perhaps a pity, but its appeal to basic physical ideas rather than to mathematics makes it a superb addition to the literature. The other chapters by Casey and Pearson and by Curtis are of intermediate style, but nevertheless are well written and complete the area being covered.

This volume, although inevitably already somewhat dated, is most welcome, but because of its high price will probably be restricted mainly to libraries.

R. C. Newman

Gauge field theory

Gauge Theories of Weak Interactions. (Cambridge Monographs on Mathematical Physics). By J. C. Taylor. Pp. xv+167. (Cambridge University: Cambridge, London and New York, February 1976.) £10.

FOR more than 25 years quantum electrodynamics (QED) has withstood the rigours of time and increasingly sophisticated experiments, and it remains *the* most successful physical theory yet formulated. Its language is relativistic quantum field theory, and it has always suggested that other interactions of elementary particles should be described in this way. The weak interactions (typical of which is β decay) looked an especially good bet since it was anticipated that a perturbation approach would be even more accurate than QED. All earlier attempts to implement such a programme, however, foundered on the rock of 'renormalisability'. That is to say, the higher order terms in the perturbation series were incalculable because of (unrenormalisable) infinities. The development of the gauge field theories in the last few years has overcome this problem, and at the same time shown how the weak interactions may be unified with QED. This latter feature is necessary in any case, from a practical point of view, since most known weak interactions involve charged particles which interact with each other electromagnetically whether we like it or not.

It is the view of most particle physicists that this renaissance of field theory is here to stay, and this provides the motivation for John Taylor's excellent book. It is not enough to know that one can in principle calculate higher order effects in such theories, one still has to know how to do it; and this requires mathematical techniques not yet in the repertoire of most practising physicists. The technicalities of the quantisation of gauge fields using path-integral formalism and Fadeev–Popov 'ghosts' are all here. There are chapters on the renormalisation of gauge theories using the Ward-Takahashi identities and dimensional regularisation, together with more familiar material on the construction of the Weinberg-Salam model and other general models. I particularly liked the chapters on spontaneous breaking of global and local symmetries.

All of this is lucidly explained, and, even more astonishingly, in only 150 pages. This book is a 'must' for all theoreticians. I anticipate that the material will soon be in postgraduate courses, so perhaps CUP can bring out a student edition. I can pay the book no higher compliment than to say that it carries on where Bjorken and Drell leave off, and at the same high standard.

D. Bailin

Plant diseases

Plant Pathogenesis. Advanced Series in Agricultural Science, Vol. 2. By Harry Wheeler. Pp.x+106. (Springer-Verlag: Berlin, Heidelberg and New York, 1975). DM39; $16.

THE Advanced Series in Agricultural Sciences has as a basic objective the integration of theoretical and technical approaches to agriculture. Volume 2 of the Series deals with pathogenesis, described as the sequence of events that occur during the development of a disease, and is based largely on diseases of higher plants caused by fungi and bacteria. A short chapter on concepts and definitions, important for setting the scene in a book of this type, is followed by a review of mechanisms by which pathogens infect, colonise and damage plants; this and other chapters emphasise the early interactions which are likely to be critical in deciding whether or not disease will develop. The third chapter complements the second by describing the reactions of plants to pathogens and does so under the headings structural, functional and metabolic. There follows an account of disease resistance with emphasis on its induction by pathogens and on the role of phytoalexins. Chapter 5 on the Genetics of Pathogenesis deals briefly with genetics of interactions between pathogens and their hosts but also considers at about the same length the biochemistry of the specificity of these interactions. The last chapter of a few pages speculates on the nature of the response of protoplasts to pathogens. There is a good list of about 200 references and a short index.

The author has ranged over much of the field of physiological plant pathology and has presented interesting and thought-provoking assessments of many different problems which should attract much research by plant pathologists and, it is to be hoped, increasingly by scientists from other disciplines. To get the best from this book the reader needs to know a fair amount about the many and various subjects which are reviewed and discussed. This, however, should not in any way deter the many others who will find in this book a very readable and informative account of most of the basic problems in plant pathology that relate to how microorganisms cause disease in plants or, more often, do not do so.

R. K. S. Wood

nature

April 29, 1976

Messages from the minefield

To attempt to comment on salary levels of scientists is to venture into a minefield. Off in various directions are snares such as historical precedents, parities between government, universities and industry, relations to the cost of living index, and rivalries, even jealousies, between different professions. The appearance of the latest remuneration survey of the Institute of Physics (*Physics Bulletin*, April 1976), however, presents a good opportunity to review the way that physicists are paid. With severe and externally imposed restraint on increases in income this year, and probably next, now is also a good time to look at a scene relatively uncluttered by salary negotiations.

The Institute of Physics has carried out salary surveys for more than 20 years, and although there has been considerable variability in the method of reporting, it is possible to see general trends. The table shows the median salary for Fellows of the Institute (the top grade; roughly 20% of the total membership). Where this figure has not been explicitly published, an attempt has been made to extract it from the statistics available. The median for the top grade may not be the ideal pointer, but it is the only one easily available, and nothing seen in other data gives the impression that it is out of line with other trends.

Date of survey	1951	1953	1956	1960	1964	1967	1971	1974	1976
Median salary of Fellows (£)	1,400	1,550	1,950	2,350	3,300	3,600	4,620	5,850	8,260
Median in 1974 (£)	3,800	3,900	4,300	4,800	6,000	5,800	6,000	5,850	5,550

The table also contains salaries converted to January 1974 levels by use of the cost of living index. Purists will no doubt shudder at the misuse of statistics: no allowance has been made for the progressive nature of income tax, and the cost of living index is only a helpful guide to those whose salaries do not differ too much from the national average. As a result the contrast between the 1950s and the 1970s is probably not as marked as it seems. Nevertheless, two points can be made: that the rewards of being a scientist grew steadily until the mid-1960s, and that since then the rewards have barely held their own.

For the past year or two we have all been rediscovering industry, relearning that the country cannot function without a productive industrial sector, attempting to help industry by increasing university links, and so on. But figures from the survey show how unattractive industrial salaries look in comparison with government and university pay. In 1974 the median salaries for all the institute's membership (not just Fellows) were £3,400 in industry, £4,420 in university, £3,500 in central government. By 1976 industry and university medians had moved roughly with the cost of living to £5,000 and £6,300, while central government had risen all of 70% to £6,000.

A cross-check on these last figures can be found in the most recent remuneration survey of the Institute of Electrical Engineers for January 1976. This confirms that median salaries in central government, the nationalised industries and universities are more than £1,000 higher than those in industry. No doubt there can be bickering over fringe benefits, security of tenure, relative qualifications, pension schemes, and so on, but the message is clear enough: the sector of the economy that is looked upon to solve our present economic woes cannot match the salary rewards that are available elsewhere; and with pay restraint this balance is unlikely to be redressed for some years. Historically, salaries in the three spheres of government, university, and industry have never got so far out of line as this. Does the government realise the consequences, including that of damping mobility out of the public sector?

Concorde's circadian conquest

"BUSINESSMEN interviewed as they disembarked at Heathrow Airport recently after a British Airways Concorde flight from Bahrain had one thing in common—they felt alert without any suggestion of 'jet lag'."

So runs a recent British Airways advertisement for Transatlantic Concorde. The operation is about as meaningful as being assured by the departing clientele of a pub at closing time that they are not suffering from a hangover.

There is no precise definition of what jet lag is, but if you gave those businessmen at Heathrow time to think for a bit they would probably perceive through the haze of Chateau Brane Cantenac '70 that they couldn't know whether they were jet lagged until they had found out whether their internal clocks were waking them up at the witching hour of 3 AM the following morning. And that will happen, Concorde or 707. But no doubt in their haste to get back to their office they would settle for a definition of jet lag as the way they felt on emerging from the plane. Besides, you've got to justify the elevated price by something besides the smoked salmon and peaches in kirsch.

Now all this is as harmless as claiming that a certain dogfood prolongs active life or a certain sauce is 99.46% pure. But wait until the businessmen are being enticed to fly to a day's work in New York and to return to London the same day. British Airways won't be interviewing them at Heathrow about jet lag, because they'll be travel-weary. They'll be rung up the following morning in their office and asked did they wake at an unearthly hour. Of course they didn't—Concorde cuts jet lag either way. □

Coal comfort for Britain?

Allan Piper reports on the fortunes of the British coal industry, reflected in the recent controversial decision to develop the Selby coalfield

Photo: WHO

Coal face

PERHAPS the most important point to be made about the plans to open up the Selby coalfield is that just three years ago any idea that the National Coal Board (NCB) might invest up to £400 milions in a single new venture would probably have been dismissed as economically senseless. In steady decline since the 1950s, the industry seemed by 1973 to be plunging inexorably to extinction, pulled by the ever tightening purse strings of successive governments intractably committed to oil and nuclear power by the strategy laid down in a 1967 White Paper on Fuel Policy. But the hiccough that arrived with the Arab oil crisis forced another look at the shape of coal's future—and the Selby project has become, perhaps deceptively, the banner heading coal's march back to prominence in Britain's energy strategy.

If all goes according to plan, the first Selby coal will arrive at the surface within four years, and by 1985 the new field should provide 10 million tons annually. But while the Selby scheme is ambitious enough itself, its real significance lies in its role as part of a wider "Plan for Coal" developed to boost sagging production levels over the next ten years. Quick to realise the opportunities presented by the fuel price adjustments of recent years, the NCB along with government and union officials formulated the ten year plan back in 1974 in an effort to stabilise and expand the British coal industry.

The aim is to introduce 42 million tons of new capacity by 1985, thus outstripping the decline of exhausted reserves and boosting production from last year's 125 million tons to an annual level of 150 million tons. Though almost half, and possibly more, of the new capacity is expected from new mines such as those on the Selby field, an equally significant amount will be brought out from pits now scheduled for extension and modernisation. And with exploration programmes proving around 500 million tons of new workable deposits each year (some even as far south as the edge of the London Basin), supplies look safe for at least another century at existing extraction rates.

In support of the expansion drive, the NCB has already begun stepping up its programme to introduce new mining equipment and techniques, and mining research is now firmly focused on the development of remotely controlled underground machinery. Initial estimates put the necessary research and development expenditure at £33 millions up to 1980. The increase in mechanisation will not see a reduction in the existing work force: the NCB view is that recruitment must average around 28,000 a year—8,000 a year more than during the cutbacks of the last decade—just to stabilise output levels. By the time target levels are achieved in the mid-1980s the industry should maintain a stable work-force of 250,000, with a hoped-for productivity growth in excess of 4% a year.

The massive investment programme at the heart of the ten year plan was put at an estimated £1,400 millions at 1974 prices, a figure which included £600 millions in new investment. These figures are already being revised to £1,800–£2,000 millions and £1,000 millions respectively because of inflation, and the NCB might even be looking for special subsidies in the future if it cannot raise the money itself. Significantly, though, the coal industry was one of the few areas to escape the heavy spending cuts announced in a recent White Paper on Public Expenditure; NCB borrowing powers have been extended from £700 millions to £1,100 millions under the National Coal Board (Finance) Act, and a further increase to £1,400 millions awaits House of Commons approval. The NCB has also received strong European support through its membership of the European Coal and Steel Community (ECSC). Since Britain joined the EEC in 1973, the Community has approved loans for the NCB totalling £143 millions.

While the new flow of cash is crucial to the revitalisation of the industry, the UK Energy Secretary, Mr Anthony Wedgwood Benn, has not been unmindful of the need for comfortable union-management relationships. He admitted that union thinking influenced his recent decision to reappoint Sir Derek Ezra as NCB Chairman when his present term ends in July, and actually published the text of a letter seeking union advice on new appointments. Ezra is regarded favourably by the unions within the industry; his period of office has so far spanned two major national coal strikes, but he has managed to remain on consistently good terms with them. Last year a deficit of £112 millions was turned round to a profit of £34 millions, and last week Ezra gave estimates that the NCB broke even in 1975–76. His new term will run to 1979, by which time the ten year plan will be well under way.

All of which seems encouraging enough. Certainly with such key issues as finance and industrial relations apparently resolved, the coal industry is anticipating a rosy future, with coal occupying a central role in any long term energy strategy. The large demands of the British steel industry are expected to rise with the next economic upturn, and recent progress in the development of high-grade coking coal may curb future demands for imports. Even more important to the NCB is the judgment that by the mid-1980s nuclear and natural gas resources can between them fulfill considerably less than half of Britain's annual energy requirements of between 400 and 500 million tons coal equivalent. And while much of the shortfall, which could be as high as 370 million tons coal equivalent, will be met by indigenous and imported oil, competitive coal prices, the NCB argues, should ensure demands for its entire output, particularly as cheap imported coal is expected to have become unavailable.

But whether or not that prognosis holds true hinges largely upon the NCB's largest single customer, the Central Electricity Generating Board (CEGB), which last year burned 67 million tons of NCB coal in its power stations (alongside which the South of Scotland Electricity Board consumed a further 4 million tons). And here the critical problem arises. The CEGB is showing a marked reluctance to commit itself to coal-fuelled generating power while oil, gas and nuclear options remain available. One reason seems to be concern at the NCB's record of efficiency. But another is escalating price rises. The NCB, as a member of the ECSC, remains unfettered by national pricing policy, and over the past two years coal prices have rocketed by an alarming 170%; the latest 15% increase adds an estimated £150 millions to the CEGB fuel bill, pushing it up to a colossal £1,600 millions a year. The CEGB chairman, Arthur Hawkins, was sharply critical: "The coal board", he said at a February meeting with Ezra, "might at least have invited its biggest customer to discuss at the highest level the serious implications of the increase, not only for electricity but for coal's prospects in the power station fuel market".

Although taking the point, Ezra's difficulty is that his hands are virtually tied. Confronted with powerful unions, the NCB finds almost half of its production costs are wage bills, while over half of present annual production is won unprofitably from older pits, using outdated machinery and production methods. The increasing volume of reserve stocks piling up at pitheads and power stations around the country adds to the problem: reserves are now standing at 30 million tons, worth £500 millions in realisable income, and costing the coal board £3 a ton in interest on borrowed capital each year. Last year excess sales over running production reduced the stockpiles by less than 10%, but it is a measure of the severity of the situation that before the NCB borrowing ceiling was raised last month, requirements rose above the earlier limit by £75 millions, an excess that had to be provided from government contingency funds.

Success for the NCB plan, then, is to an important extent contingent upon CEGB agreement to convert oil and gas-fired power stations to coal. In proposing an 11 million ton increase in coal burning by the CEGB over the next two years, Ezra has pointed out that a switch from oil and gas to coal could save the electricity industry £3 to £4 on each ton of fuel, and the country £250 millions on the balance of payments. The idea reportedly has the backing of the Department of Energy, but Hawkins has dismissed it as "quite ridiculous" at a time of expenditure cutbacks in the nationalised industries and falling consumer demand (which last year dropped by an estimated 5%). Further, aside from conversion costs, Hawkins has also to consider the heavy national investment in North Sea oil and gas.

As for the less immediate outlook, NCB hopes are similarly overshadowed by CEGB reluctance to order new coal-fuelled power plant. Though the coal-fired, 2,000 MW installation at Drax in Yorkshire has just reached completion, 5 of the 12 remaining installations still under construction in Britain are nuclear powered, and 6 are oil-fired; only the 1,500 MW Aberthaw B in South Wales, due on-line around the end of the decade, is designed to burn coal. In addition, CEGB last month announced that forward ordering plans, already unlikely to favour the NCB, may well be revised downwards following the most recent demand forecasts. These indicate that consumer demand for the winter of 1982–83 will not rise above a peak of 52,000 MW, a surprising 2,000 MW down on last year's forecast for 1981–82, and only around 25% —or 3.5% a year—over current demands.

The CEGB has nonetheless made a couple of moves towards raising its requirement for NCB coal. Imports, which last year totalled 4.5 million tons, are to be cut. Hawkins recently announced that import contracts undertaken during 1973–74 to offset the then prevailing shortage of British reserves are to be run down over the next financial year. And CEGB demand for British coal last year topped the previous year's level by 3 million tons, while oil consumption was cut by almost a third to only 14 million tons coal equivalent. This turn-round indicates that if the price, quality and demand is right, the CEGB's 111 coal-fired installations could burn more coal than at present—in fact the requirement could rise as high as 90 million tons of coal a year, a figure more compatible with the aims of the ten year plan than the existing level of 67 million tons. The present shortfall occurs simply because demand levels above the early morning baseline of 22,000 MW are now met by oil-fired generating power in preference to coal power. But once the Plan for Coal gathers sufficient momentum, increasing productivity and cutting production costs, the NCB could swing that method of priority operation to its own advantage, leapfrogging coal-fired generating power above the oil and nuclear options.

Should that possibility not prove successful, export markets offer little prospect of an easy solution to the glut. In north-western Europe, the only realistic potential outlet, British coal will face competition not only from low-priced North American exports, but also from Polish coal maintained at continually depressed "administered prices" which undercut competition.

The £400 millions Selby project, however, in keeping with the optimistic front it presents to the outside world, remains for the most part unscathed by the wider complications, though critics of the ten year plan may disagree. The Main Barnsley Seam, stretching for 110 square miles beneath the Vale of York and an amenable three or so metres thick, will provide almost half of the new annual output by the mid-1980s. Geological conditions are sufficiently favourable to allow extraction using "drift mining" techniques, involving production through a single sloping tunnel rather than multiple vertical shafts. Production is expected to begin within four years, and when full operational capacity is achieved by the mid-1980s a maximum of 52,000 tons of coal a day will travel by conveyor to the surface, providing the nearby coal-fired power stations at Drax, Eggborough and Ferrybridge with continuous fuel supplies by rail.

Ezra says Selby will produce "the coal which, with North Sea oil and gas, is going to make Britain the only main nation in Europe which will be self-sufficient in energy by the mid-1980s". With the NCB also engaged on large research programmes in pyrolosis and liquefaction techniques, and British and European research efforts continuing in the areas of fluidised bed combustion and coal gasification, the future does seem promising. But there are still uncertainties to be removed if the turn-round is to materialise. □

Coal cutting machine

The threat to peer review

Colin Norman reports from Washington on a series of events which provides a vivid example of the difficulties sometimes encountered by scientists engaged in research on controversial topics

IN A move believed to be without direct precedent, the House of Representatives on April 13 voted to shut off funds for a scientific research project because a powerful Congressman believes the project is scientifically unsound and morally unacceptable. A number of scientists have charged that the vote, if upheld in the Senate, constitutes a worrying challenge to academic freedom.

The project is certainly controversial. An attempt to determine the effects, if any, of marijuana on human sexual responses, it attracted considerable press attention when it was approved last year, and it has stirred up various expressions of moral outrage. Nevertheless, the research has been judged scientifically meritorious by committees of scientists expert in the field, endorsed by the federal government's top advisory committee on drug abuse, and been subjected to a searching inquiry by the government's senior health officials. If Congress does abort the project, the move is certain to be interpreted in the scientific community as political interference with the painstaking peer review process used by virtually every government agency to determine the relative merits of research proposals.

The project is a two-year research effort to be conducted by Dr Harris Rubin, a behavioural scientist at Southern Illinois University Medical School, Carbondale. Rubin's research protocol notes that though there have been many verbal and subjective reports indicating that marijuana has a substantial effect on human sexual behaviour, those effects and the drug's mode of action have not been objectively evaluated. His project involves giving measured doses of marijuana to volunteers and then measuring their physical responses to visual stimuli such as erotic films. Part of the expriment also involves monitoring blood levels of various sex hormones, such as testosterone.

Rubin applied for a grant from NIH for his project in May 1974, and his application was passed on to the National Institute on Drug Abuse (NIDA). It was reviewed for scientific merit by a peer review committee in the usual manner, and two well known behavioural scientists — Dr Joseph Brady of Johns Hopkins University and Dr Jonathan Cole of Columbia University—conducted a site visit and discussed the application with Rubin in January 1975. The peer review committee subsequently approved the project as being scientifically valid, and NIDA decided to fund it in June 1975 with a grant of about $121,000 over two years.

It was then that Rubin's troubles began. A routine announcement of the grant by Southern Illinois University was picked up by the press, several accounts of the project were published, and it was attacked in the *St Louis Globe Democrat* as "tax-paid debauchery". Then Senator William Proxmire, the colourful Senator from Wisconsin who has frequently denounced scientific research which he considers trivial or unsound, took the project in his sights and fired off a press release calling it "one of the most shocking examples of the 'federal love machine' I have ever found". That attracted considerable attention from the media.

While that furore was beginning to break, Rubin applied to the Department of Justice for a grant of immunity from federal prosecution, normally a routine procedure for scientists conducting approved studies with illicit drugs. The application turned out to be far from routine, however, for the Justice department sat on his application for six months, and in January this year it asked the Secretary of the Department of Health, Education and Welfare (of which NIDA is a part) to reevaluate the project and determine whether Rubin is suitably qualified to conduct the research. According to HEW officials, the Justice Department's action is unprecedented.

The reevaluation was carried out on March 11 at a closed meeting attended by the head of the Food and Drug Administration, the Assistant Secretary for Health, the head of NIDA, other government officials and a number of outside scientists—probably the most high-powered committee ever to consider a single grant application. According to HEW sources, the meeting resulted in a recommendation to David Matthews, Secretary of HEW, that the project continue to be supported. Matthews has not yet acted on the recommendation. Similarly, on February 11, the NIDA National Advisory Council, a committee composed of scientists and lay members, unanimously passed a resolution urging Secretary Matthews to support the project.

Rubin has received nearly half of the grant from NIDA, but his project is in limbo until he receives his grant of immunity from the Justice Department.

Meanwhile, Congress entered the picture largely through the work of Representative Robert Michel, the House Republican Whip, who represents Peoria, Illinois. Michel, a powerful member of Congress, is the most senior Republican on the appropriations subcommittee which handles HEW's budget. He has denounced Rubin's project on several occasions, calling it "offensive to the sensibilities of most Americans", and he has twice quizzed HEW officials about it.

Michel made his decisive move against the project by writing into a supplemental appropriations bill containing funds for a number of different agencies, a provision cutting off support for Rubin's research. The bill was passed by the House on April 13 with scarcely anybody noticing Michel's handiwork. The bill is now being considered in the Senate, and a number of scientists have already written to their Senators to ensure that the provision doesn't slip past that body unnoticed.

Though there may indeed be questions about the utility of Rubin's research proposal, the fact is that the project has been endorsed by Rubin's scientific peers, and it has been approved after a painstaking review procedure which is a basic ingredient of federal support for science. If Congress were to overturn the funding decision, it would be an unprecedented step. Dr Joseph Brady, one of the chief reviewers of the project, last week called the House vote "outrageous", and pointed out that the review procedure "was a model of the way the peer review system should and can work".

Dr Robert DuPont, the head of NIDA, noted in a telephone interview last week that "Congress has the perfect right to assert itself in terms of how federal money is spent", but he added that "if Congress is going to reverse the considered judgment of the peer review process, I think it should be extremely cautious about setting a precedent".

As for Rubin, he said that he is "very disappointed" by the House vote, and he argued in a letter sent to several of his colleagues that if the Senate follows suit, "the Congress will be overruling the peer review process and politicians rather than scientists will be determining what specific research projects will be conducted . . . the danger to scientific and academic freedom in this precedent is enormous". ☐

USA

AFTER several months of negotiations richly laced with minor squabbles, key Senators and Congressmen have at last reached agreement on a bill to reestablish a science policy office in the White House. Final Congressional approval of the measure is generally deemed imminent, and the office will probably be off and running by the end of May—nearly three and a half years after Mr Nixon, arguing that he no longer required the services of a full-time science adviser, dismantled the former White House office of Science and Technology.

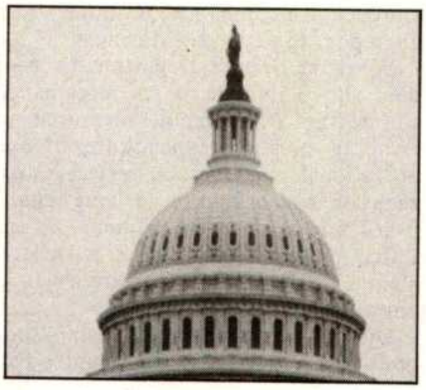
The bill has been in limbo for weeks while Congressional staff members and their bosses have been trying to work out a compromise between three conflicting versions of the legislation—a modest proposal put forward last June by President Ford, a slightly more ambitious measure approved in November by the House and a more substantial bill passed by the Senate in February. A compromise was finally struck in mid-April, and its swift approval by the House and the Senate is virtually assured.

It would establish a small office of Science and Technology Policy (OSTP) in the White House, headed by a director who would also double as the President's Science Adviser, and four associate directors.

Though it has always been assumed that the office would provide advice and assistance to the President and other White House bodies on matters involving science and technology, its exact role and scope have been in dispute. The compromise version of the bill specifies that the Director of OSTP will be a member of the Domestic Council, a top-level White House policy-making body for domestic affairs, and he will also be a statutory adviser to the National Security Council, the powerful defence and foreign policy committee formerly headed by Henry Kissinger. Those roles will at least ensure that the office will have a voice in both civilian and defence policy.

As for budgetary matters, the bill simply requires OSTP to assist the Office of Management and Budget in preparing the Administration's annual budget request, a role which falls far short of the powerful voice in fiscal matters for which many scientists had hoped. The bill does, however, provide two other mechanisms for OSTP to influence budgetary policies. First, it requires the office to prepare a five-year forecast of emerging national problems and provide a set of programme options to every government science agency at the start of each budget cycle. Secondly, it requires OSTP to publish an annual report on federal science and technology, a mechanism which should allow the office to make public its views on the budgetary state of science.

Another important provision in the bill will create a President's Committee on Science and Technology, consisting of between 8 and 14 prominent people, to conduct a two-year survey of the entire federal science and technology enterprise. The President will have the option of making the committee into a permanent body when it has completed its study.



Though the office has yet to be formally established, there is considerable speculation about who President Ford will choose to head OSTP. According to Congressional and Administration sources, there are three potential candidates: Dr Simon Ramo, Vice President of TRW Inc., Dr William O. Baker, President of Bell Labs, and Dr H. Guyford Stever, Director of the National Science Foundation, who now doubles as the President's part-time science adviser.

- The Nuclear Regulatory Commission (NRC) has, as expected, denied a petition seeking a drastic tightening of federal standards governing permissible levels of exposure to plutonium. The petition, filed in February 1974 by the Natural Resources Defense Council (NRDC), was based on the so-called "hot particle" theory which suggests that tiny particles of inhaled plutonium pose a severe health hazard because they lodge in the lung and deliver a prolonged, intense dose of radioactivity to the surrounding tissue. Since present plutonium exposure standards are based on the average dose of radioactivity to the whole lung, rather than to tissue around inhaled particles, the NRDC petition argued that the standards are too lax and should be tightened by a factor of 115,000. The matter has been a subject of considerable debate.

The NRC turned the petition down, and denied NRDC's request for public hearings on the matter, however, because it found that "scientific evidence does not support the technical position upon which the NRDC petition is based". Drawing upon several studies, including reports by the UK Medical Research Council and the former Atomic Energy Commission, the NRC believes that the hot particle theory is flawed in several respects and does not provide a valid basis for establishing exposure standards.

NRDC has also petitioned the Environmental Protection Agency (EPA) with a similar request. EPA is awaiting a report on the hot particle theory from the National Academy of Sciences (expected in about a month) before issuing its reply.

- Following much prodding from Congress and a good deal of agitation from a number of independent expert groups, the Energy Research and Development Administration (ERDA) last week placed increased emphasis on energy conservation in its overall plan for decreasing the United States' dependence on foreign sources of oil. The new emphasis is incorporated in an updated plan for energy research and development, published last week by ERDA, which spells out in some detail the agency's goals and strategies.

The plan, an expanded and revamped version of a document published by ERDA on June 30 last year, ranks the development of technologies to increase energy efficiency and conservation among ERDA's highest priorities. Unveiling the plan at a press conference, Dr Robert C. Seamans, Jr, ERDA's Administrator, said he believed it was "impossible to exaggerate the need and desirability to make more efficient use of energy".

Though the plan isn't very specific about the exact strategies envisaged for conservation, Seamans said that the agency would be putting together "some very significant projects" in the next six months. He added that conservation efforts could save up to five million barrels of oil by 1985.

In other respects, the plan is similar in balance to last year's version, placing heavy emphasis on nuclear energy, coal and production of synthetic oil and gas from coal and shale in the medium term.

As for nuclear power, the plan anticipates that installed capacity will grow from its present level of 39.6 GW to 70–76 GW by 1980, 160–185 GW by 1985, 265–340 GW by 1990 and 450–800 GW by 2000.

AUSTRALIA

Filling the information breach

From Sydney, Peter Pockley looks at Australia's science policy in light of the Science Department's recent Annual Report

SOMEWHAT dated and dented by more recent political and bureaucratic changes, the Annual Report of the Australian Department of Science and Consumer Affairs for the year July 1974 to June 1975 has now surfaced. The most visible dent on the Department is the recent removal of consumer affairs from its title and duties.

Nonetheless, this retrospective report is useful, if only for combining in one list the various activities which, under the now deposed Labor administration, had been brought under the one department. It is, however, as informative to note the omissions from this list as the inclusions, for Labor's Department of Science and Consumer Affairs exercised only partial coverage of the national science scene, a situation unchanged under the new regime. By international comparison, Australia spends in the government sector one of the highest proportions of total research funds (of the order of three-quarters). National figures are not yet available for the year in question, but a conservatively generous estimate puts the Department of Science's proportion of this expenditure (that is, funds directly controlled by the Department) at no higher than a quarter, probably less. At least three-quarters of "in-house" government-financed research was done outside the Department's control.

The most obvious organisation standing beyond the influence of the Department is Australia's colossus of research, CSIRO, with expenditure of $143 million in 1974–75. In company with the newer and smaller statutory bodies, the Anglo-Australian Telescope and the Australian Institute of Marine Science, CSIRO remained responsible directly to the Minister for Science and Consumer Affairs, and not through his Department. (Again, this arrangement did not change with the change in government in December 1975.)

While a lack of mention of CSIRO in the Department's report is strictly correct in legislative terms (indeed, if the proudly independent CSIRO had been mentioned by the Department from which they keep a cool distance, there could have been a row of epic proportions), it means, unfortunately, that the report cannot be treated by local or international readers as representative of the national science scene.

It should also be added that substantial government-financed research and development efforts are also carried on outside the responsibility of the Science Minister. Notable among these are the Australian Atomic Energy Commission, the Bureau of Mineral Resources, and the Weapons and Aeronautical Research Laboratories, who all report to separate Ministers.

What the Science Department report does show, though, is the dominance within the Department, measured in terms of staff and expenditure, of five operational research and service units, each of which had been long established under other Departments before Labor placed them under the Department of Science from December 1972 onwards.

Out of a total of 3,368 established positions, these operational units had 3,097 and the central office 271. The Bureau of Meteorology ($31.2 million), the Patent, Trade Marks and Design Office ($5.7 million), the Antartic Division ($4.5 million), the Analytical Labortories ($2.7 million) and the small Ionospheric Prediction Service ($600,000) accounted for 78% of the Department's overall expenditure of $57.2 million. If $8 million of payments to university researchers by the Australian Research Grants Committee are included, the proportion of these six activities rises to 92%. Through revenue earned, the Patent office was almost entirely self-supporting, and the Bureau of Meteorology earned over a third of its keep, the total revenue of the Department being $17.5 million.

The Department also administers civilian space projects conducted through the operations of three tracking stations for the American NASA. Of the 400 employees in this work, only 40 are Australian public servants; the $11 million cost in 1974–75 was met by NASA.

The Department can point to an apparently modest sum of $2.6 million being spent on general administration. However, the operational units had largely self-contained administrations before their transfer to the Department, and the geographical separation from Canberra of most of them had probably not allowed much in the way of reduced administrative expenses. The Department talks of developing "staff resources without any significant increase in numbers", and of units becoming "more effective as a result of reorganisation". Personalities aside, it is not surprising that friction did develop between the central administration and some of its operational outposts; naturally none of this emerges in the report.

Policy influence

The constraints on an Australian Department Head in writing a report to his Minister are such that only the blandest information on matters of policy and planning is presented. For instance, Sir Hugh Ennor's report does note the formation of the Interim Australian Science and Technology Council (ASTEC) as "an important, but not exclusive, source of advice to the Government". The report adds that "the Department's work will both supplement and complement ASTEC;

AAT change

THE 3.9-metre Anglo-Australian Telescope (AAT) on Siding Spring Mountain, New South Wales, is to have a new Director in September. He is Professor Donald Morton, of Princeton University, and he succeeds Professor E. J. Wampler who left at the end of last month to return to the Lick Observatory in California. Wampler's appointment was for a period of two years ending in October, but by "mutual agreement" he has withdrawn early—mainly because he considered complete his task of seeing the AAT commissioned, but also, it is believed, because he was not completely happy about future prospects for the telescope at a time of economic stringency.

Not that the telescope is lacking funds, according to Professor Vincent Reddish, of the Royal Observatory Edinburgh, who sits on the AAT Board. Its present annual budget is about £1 million, equally financed by the UK and Australia, and, says Reddish, all the indications are that it will continue to be well looked after by the two governments as an important ongoing project.

One of the most interesting aspects of the AAT is the way in which it has been operated hand-in-glove with the UK 48-inch Schmidt Telescope, located a few yards away on the same mountain. The Schmidt is essentially a survey instrument, and on most days AAT astronomers are to be seen in the Schmidt building poring over the most recent plates to find astronomical objects worthy of more detailed examination by the AAT. Much useful work on the spectrometry of faint galaxies, and the dynamics of groups of them, for example, has been done by the AAT in tandem with the Schmidt.

Roger Woodham

in particular, it will act as an agent for ASTEC in obtaining and supplying data in conducting or commissioning studies and in carrying out those liaison and other tasks both within and outside Government that it is favourably placed to undertake". As far as the Department was concerned, the demarcation lines in the highly competitive business of influencing the government are kept nicely fuzzy. All this, of course, may be water under the bridge when the Liberal Government's current review of ASTEC is publicised and implemented.

In support of its own advisory role, the Department has pursued its data collecting activities. The long-running Project SCORE (Survey and Comparison of Research Expenditure) first surveyed expenditure and manpower in Australian research and development for the financial year 1968–69; the results were published in 1972 and 1973. Project SCORE is now reported to be "well underway" on "two-yearly intervals" and using OECD bases for statistics "to be sure that Australian figures may be compared with overseas figures".

The day after the report's period of survey concluded, that is, July 1, 1975, the 555-strong Patent Office was removed from the Department and transferred to the Attorney-General's Department (more recently still it has found another home in Business and Consumer Affairs).

It is easy for outside observers (and most insiders, too) to be bamboozled by the dispersed nature of Australia's scientific organisation. This is not, however, an argument for centralised control, but for at least one government sponsored effort to document in assimilable form the standing and overall progress of science on a national scale. The Science Department's report cannot claim to do this, nor to be fair does it claim to do so. Yet, not many scientists in Australia are more than dimly aware of what is going on in fields outside their own speciality. The interested public and politicians find the task equally tough, although they are helped by some very informative programmes on ABC radio.

The only organisation in Australia which has had the drive and capacity to fill this information breach is the interim ASTEC, but it is still on ice while the government reviews its aims and operations. If, however, ASTEC is allowed by Prime Minister Malcolm Fraser to survive in anything like its form under Labor which would make public reporting central to its functions, then the prospects of the scientific community and general public alike catching and keeping up with the hare of Australian research are quite bright. Or, is it a tortoise? ☐

USSR

Give and take

April, traditionally an auspicious month for Soviet scientists with the announcement of the Lenin Prizes, has this year brought an ominous picture for those out of favour with the establishment: two dissident scientists on trial, continuing harassment and repression of refusniks, and Academician Andrei Sakharov briefly held with his wife in police custody. Vera Rich reports

THE recent "Omsk incident" involving Academician Sakharov marks a new development in the Soviet authorities' campaign against him—the loss of his personal immunity. It happened when Sakharov and his wife Yelena tried to attend the trial of Mustafa Dzhemilev, a young agronomist who has become the leader of the movement for the repatriation of the Crimean Tartars, deported *en masse* to Siberia in 1944.

"Political" trials in the Soviet Union are not normally open to the public, but Sakharov did sometimes manage to enter the courtroom as a friend of the accused—in the case of the mathematician, Dr R. I. Pimenov, in 1970, for example. By 1972, however, when Vladimir Bukovskii was on trial for having sent to the West detailed reports on Soviet misuse of psychiatry for political ends, Sakharov was no longer admitted, protesting instead outside the court. Now he and his wife have been prevented from doing even this.

According to Sakharov, who says two official TASS bulletins on the incident are false, plain-clothes KGB men prevented the friends and relatives of the accused from entering the courtroom, using "rude physical force", directed "in particular" at Sakharov and his wife. In an "immediate reaction" to the "mockery of the feelings of friends and relatives, the mockery of the law, the whole tragic circumstances of this case and other political cases in our country", Sakharov said he hit in the face "one or two KGB men" and a civil policeman who was acting on KGB orders. The Sakharovs were removed to police headquarters, where Sakharov made a statement claiming that the police were acting "on the side of the law-breakers". The next day, the Sakharovs tried once again to protest against the exclusion of the Dzhemilev family from the court, and again were taken to police headquarters.

Sakharov stresses that the civil police treated them "correctly", and says that earlier reports to the contrary were due to a bad telephone connection to Omsk. He denies vehemently that he created a disturbance in the courtroom, as TASS had claimed. This was quite impossible, he says, since "three ranks of KGB men" prevented them even getting near the court. He admits that the possibility of criminal charges against himself is "not excluded".

Sakharov's attempt to focus world attention on the Dzhemilev trial in Omsk, a city closed to foreign journalists, came at the same time as his friend and close associate in the human rights movement, physicist Andrei Tverdokhlebov, faced similar charges of anti-Soviet activity in Moscow. Sakharov felt that the location of this trial in the capital, and the fact that Tverdokhlebov's membership of the illicit Moscow group of Amnesty International makes him a well-known figure, would ensure publicity abroad, whereas the Dzhemilev trial might pass unnoticed without his presence. Tverdokhlebov was sentenced to 5 years' exile; Dzhemilev received 2½ years in a strict regime labour camp.

As for the general position of dissident scientists, this shows little sign of improving. The Kiev seminar for Jewish refusnik scientists, one of the many off-shoots of the original Voronel seminar in Moscow, and led by physicist Vladimir Kislik, has been shut down. Mark Azbel, who now runs the Voronel seminar, has once again been refused a visa for Israel. And Professor Veniamin Fain, one of the very few activists and refusniks to be allowed to continue in his academic career, has now been dismissed from his post at the Institute of Solid State Physics in Moscow.

Sakharov has meanwhile accepted an invitation to become a member of the executive committee of the Campaign Against Psychiatric Abuse (SAPA), the British section of the International Initiating Committee against Misuses of Psychiatry for Political Purposes. ☐

Photo: AP

Andrei Sakharov

IN BRIEF

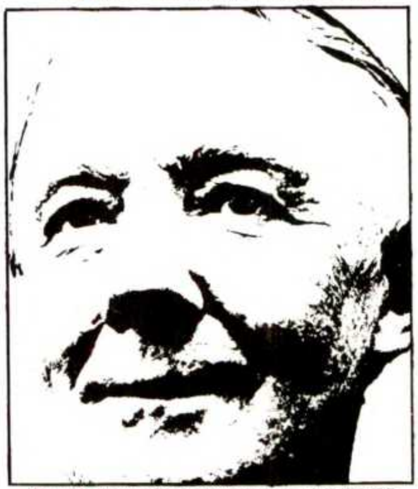
Uranium meetings

The first ever talks between consumers, processors, and producers of uranium have been called to coincide with the annual general meeting of the UK Uranium Institute in London on June 15. The Institute, a "think tank" set up last year by uranium producers, admitted electrical utilities and nuclear fuel reprocessors in January, and at the June meeting representatives are expected from Western Europe, Australia, Japan, North America and South Africa. They will discuss the physical and commercial factors influencing the uranium market and the issues they raise for the industry. In Vienna earlier this month, delegates from 38 states attending an International Symposium on the Exploration of Uranium Ore Deposits, arranged jointly by the International Atomic Energy Agency (IAEA) and the Nuclear Energy Agency (NEA) of the OECD, heard that the discovery rate must increase from around 80,000 short tons a year to nearer half a million by the turn of the century if projected demands are to be met. The IAEA and NEA are now looking at the need for "a more permanent group of experts" to consider the best directions for research and development in exploration techniques.

Indian population measures

In an effort to combat the country's population growth, India's Minister of Health announced earlier this month an increase in the minimum marriage age, from 18 to 21 for men and from 15 to 18 for women. Compulsory national sterilisation will not be introduced for the moment, though individual states will be allowed to enforce their own sterilisation measures for couples with three children. Incentives for voluntary sterilisation will include Government payments to couples of up to 150 rupees (around $17); schemes introducing favourable priorities for jobs, accommodation and loans will be left to individual states. About 8% of federal government aid to states will be directed towards family planning; the amount states will receive will be based on 1971 population figures, as will the number of seats they hold in Parliament. Three states — Maharashtra, Haryana and Punjab—have already drafted legislation for compulsory sterilisation. India's population, estimated at 600 million, could top 1,000 million by the turn of the century if it continues rising at the present rate of 1 million a month.

Zoo celebration



KENNETH MELLANBY

APRIL 29, 1976 is the 150th anniversary of the historic meeting at which Sir Stamford Raffles proposed the formation of the Zoological Society of London, best known to the public because it established the London Zoo. Even then, however, the creation of a menagerie came second to "the advancement of Zoology and Animal Physiology" in the objectives of the society. For the last 150 years the society has continued its scientific work in addition to maintaining the collections of living animals in Regent's Park and, since 1931, at Whipsnade in Bedfordshire.

A visit to a zoo is generally looked upon as a harmless form of recreation, though there are those who voice moral objections to the display of captive animals. In the past, some zoos kept animals in unduly restricted cages, fed them on inadequate diets, and paid scant heed to their welfare. As a result disease was rife and survival short. In addition, populations of rare wild animals were often depleted or exterminated when collectors killed many more than they shipped back to maintain the collections. The Zoological Society of London was, from the first, aware of these dangers, and tried to improve the condition of their animals by studies of their physiology, nutrition and behaviour. Attempts were also made to breed as many as possible to reduce the pressure on the wild stock.

Even in 1877, when the Regent's Park zoo was being planned, efforts were made to house the animals properly: the architect employed by the society, Decimus Burton, divided his efforts between designing the Atheneum Club for the literary and scientific leaders of the period, and creating a house for the giraffes in the zoo. The club has changed little, but standards of animal accommodation have improved considerably.

Zoos are used for systematic teaching of zoology as well as to show visitors the variety of the world's animal life. British children learn about the fauna of other lands, and many Africans see lions and other species seldom visible in their homelands, though recently the opening of zoos in Africa has changed this. Large crowds at the University zoo at Ibadan in Nigeria can admire a healthy pride of African lions, possibly unaware that they derive from a lion denoted by Longleat in Britain and a lioness from Dresden in East Germany.

Scientific meetings started even before the zoo opened its gates. In April 1827 Dr Joseph Brookes lectured to a substantial audience on his dissection of an ostrich. The scientific journals of the society have continued to be published since 1830. Much of the earlier work was anatomical, using the corpses of animals which died, but there have always been studies of diet and behaviour aimed at improving the conditions of the exhibits. However, not every opportunity for research has been exploited: Sir Peter Chalmers Mitchell (best known for his efforts to establish Whipsnade) told me in 1936 how he had been approached by several ageing members of the aristocracy, who asked to be allowed to act as voluntary keepers of the larger mammals, in the belief that this would enhance their virility. It would have been interesting to have assessed the results; the society might even have gained a source of much-needed income!

Since the last war, scientific work has made further progress. The establishment of the Nuffield Institute of Comparative Medicine, the Wellcome Institute of Comparative Physiology and a modern Animal Hospital has provided unique facilities for research. These are well used, but more money will be needed if their potential is to be fully exploited. The society is now in a position to lead the world in work essential to the conservation of the many animals which are increasingly endangered by human population growth, industrialisation and pollution in their homelands. Conservationists and animal lovers need the Society today even more than they did 150 years ago.

Pure Chemistry Library
SCIENCE COLLEGE.
92, Upper Circular Road
CALCUTTA

correspondence

Genetics at the OU

SIR,—Since our veracity, objectivity and judgment have been called in question in a letter signed by Professor Pentz (April 8, page 479), which contests the criticism which we made of the genetics course now offered by the Open University, we hope you will allow us to put the record straight.

Much of the letter is polemical in the worst sense ("gratuitous insult", "prejudice", "mere opinion"). It also distorts our letter to mean what it does not say, namely, that we were attacking *all* Open University courses. In fact, we explicitly and specifically criticise the genetics course only.

The charges of falsehood made against us turn on the technicality of what constitutes "the Consultative Committee". The full Committee consisted of two members of the OU Course Team engaged in writing the course (Professors Rose and Jones), Dr C. Butler as Director of the Nuffield Foundation (subsequently replaced by the new Director, John Maddox), Professor H. Kornberg as a Trustee of the Foundation and five geneticists not involved in the writing of the course (Professors Jinks, Lewis, Pritchard, Sang and Dr Kacser). A sixth geneticist, Professor Bodmer, who was initially a member, later resigned.

It was these five geneticists who were actively engaged in meetings with the course team. In many sessions they made some far reaching criticisms and suggestions, including the proposal to reduce the coverage of the course radically (Consultative Committee meeting of October 20, 1975), but these were largely ignored. Professor Rose was the only member of the course team who attended all meetings. How much of our comments reached the rest we have no means of knowing. Eventually, and in despair, the five felt compelled to write a report to the Foundation who had appointed them. This, quite properly, was confidential and could not have included any member of the course team. All other members received a copy. The debating point that they were not the full Committee, appears to have been introduced to conceal the reality of the situation. In a final attempt to improve the course, the Chairman of the Committee and the Director of the Foundation had a meeting with Professor Rose where the substance of our suggestions was discussed with him. The most important ones had, of course, been previously discussed in Committee. None of our major suggestions were accepted by Professor Rose.

The charge of "prejudice" turns out to be that the considered opinions of five geneticists with many years of teaching experience and great interest in teaching methods are set against the reports of some students that Units 1 and 2 were "OK", "very interesting" or "very easy". We are happy to leave this charge to the judgment of our colleagues.

We have gladly given of our time and experience for an enterprise which, at the outset, appeared to us both academically and socially important. In all our endeavours we had, and continue to have, only the welfare of potential students in mind. Because of this we must re-iterate that the students are ill served by the course as it stands. It is too long, too advanced and too detailed for second level part-time students and is inadequately prepared for non-OU Institutions. It would have been irresponsible to have brushed this opinion under the carpet even if our actions caused embarrassment to a member of the OU staff. Furthermore a venture which has absorbed a very large amount of money from a charity and, as we are now informed, an even larger amount of public money, should not be immune from informed criticism. There is nothing sacred about a Genetics Course—even an Open University course—which is being actively promoted for sale to Universities at large.

Yours faithfully,

J. H. SANG
School of Biological Sciences,
University of Sussex

H. KACSER
Department of Genetics,
University of Edinburgh

R. H. PRITCHARD
School of Biological Sciences,
University of Leicester, UK

Science and the media in Canada

SIR,—In reading David Spurgeon's account of science and the mass media in Canada (February 5, page 353), I was interested in noting the abrupt injection of subjectivity in the otherwise quite objective summary, represented by the rather plaintive "how would they know?" comment, referring to popular scepticism of newspaper science articles.

I could cite many cases of science reporting in Canadian newspapers, and personal contacts with reporters, where the clear objective has been to overdramatise science stories, frequently through exaggeration, on the admitted grounds of necessity to catch the readers' eyes. Perhaps this is more acute in the case of environmental sciences (one reporter from a large daily wanted a concoction of disgusting material prepared to be photographed as an example of pollution in the Great Lakes).

Consequently, although I share Mr Spurgeon's dismay at the apparently poor job we are doing in informing Canadians of scientific matters, I must express some relief to learn of our sceptical public.

Yours faithfully,

ROBERT K. LANE
Edmonton,
Alberta, Canada

Competition 7

A PRESENT day headline for Charles Darwin's achievement might read "British scientist finds life's secret on tropical island".

A prize of £10 is offered for the best modern headline for any scientific achievement past or present. Inflated claims or deceptive packaging are welcome. The closing date for entries is June 8.

Competition 6 required a rhyming couplet to help familiarise an SI unit. M. Hammerton of Newcastle receives honourable mention for a McGonagall verse, as does P. A. Mohr of Cambridge, Mass., for his brave efforts to keep the Imperial system alive:

The 3,000 metres steeplechase
Is a two miles less one-and-a-twelfth furlongs race.

But the clear winner was John Gribbin of Brighton with:

A Pascal of pressure on top of your head
Is the same push that butter exerts on sliced bread.

news and views

Does the key fit the locks of the past?

from Peter J. Smith

FEW would now disagree with the view that the plate tectonics hypothesis has provided a most successful model of the tectonic activity now taking place at the Earth's surface. Obviously there are still many unresolved problems, not the least of which, as Helwig has demonstrated recently (*Nature*, **260**, 768; 1976), is the clarification of the processes through which the collision of plates is translated into orogeny. Nevertheless, the basic outline is there, and for the time being there is little reason to suppose that the filling in of the detail is more than a matter of practical application, however difficult that might turn out to be. As Le Pichon *et al.* (*Plate Tectonics*, Elsevier, 1973) have pointed out, the supreme advantage of the plate tectonics hypothesis is that "it is predictive and can be tested by measuring relevant parameters."

But the real problems start with the attempt to extrapolate conventional plate tectonics backwards in time. Some of the difficulties are fairly obvious. An important part of the evidence for modern plate activity comes, for example, from the horizontal and vertical distribution of earthquake hypocentres. But there is no way of directly observing past earthquake distributions; ergo, important evidence for past plate activity is unobtainable. Other types of evidence, such as that from oceanic magnetic lineations, are in principle more permanent but in practice of very little help. As everyone now knows, ocean floors have an unfortunate tendency to disappear down subduction zones, destroying most of their history with them. As most constructive ridges and transform faults occur in oceanic crust, the chances of examples of either plate boundary having survived the past 200 million years are remote.

The burden of evidence in favour of the past existence of active plates must therefore rest on the identification of examples of just one of the three types of plate boundary—the consuming margins which have some direct association with continents But in addition to the practical problems, this raises what some would no doubt call a philosophical issue. The successful identification of old subduction zones is (or, in deference to the cynics, should be) rather dependent upon there having actually been subduction zones in the past. Cady (in *Implications of Continental Drift to the Earth Sciences*, Academic Press, 1973) has remarked that not only do "the earmarks of subduction become blurred with time" but they "may be recognised only by those who believe the present to be an invariable key to the past". Thus Le Pichon and his colleagues, whilst appreciating the difficulty in testing models of older plate tectonic processes and being sceptical of attempts to do so, nevertheless claim that "there is no reason to suspect that there has been any change in the major causes of deformation of the earth during the past billion years or so". This is a view commonly expressed and appears to be based on an ingrained reluctance among Earth scientists to question the tenets of uniformitarianism, however vaguely understood. But is it a valid statement? Is there really no cause to suspect?

There is probably little cause for suspicion about the past 200 million years. The difficulties of identifying Mesozoic fossil plate boundaries are far from negligible, but it is at least evident that throughout this period the present continents were drifting, ocean floors were spreading and plates were interacting much as they are now. The most convincing example of a Mesozoic subduction zone is perhaps the Great Valley region of the western United States To the west of the Valley lies the Franciscan Formation, a chaotic mélange apparently deposited on oceanic crust But the Great Valley sequence itself, separated from the Franciscan by a long sharp thrust fault system but spanning the same time interval, is a more orderly succession of miogeosynclinal rocks overriding part of the mélange to the west and resting on continental crust in the east. As Hamilton (*Geol. Soc. Amer. Bull.*, **80**, 2409; 1969). Ernst (*J. geophys. Res*, **75**, 886; 1970) and others have argued, this is a trench-like environment probably representing an ancient site of Pacific plate subduction; and Dickinson (*Rev. Geophys., Space Phys.*, **8**, 813; 1970) has even reconstructed the position of the sloping palaeoseismic zone on the basis of potash values in the Sierra Nevada which stretches to the east.

But in respect of the Palaeozoic and Precambrian, matters may not be even this simple. Piper (*Earth planet. Sci. Lett.*, **28**, 470; 1976 and elsewhere) has shown palaeomagnetically that Africa and North America apparently acted as a single unit from 2,700 billion years ago to the breakup of Pangaea—a conclusion strongly implying, but far from proving, that Pangaea itself existed for at least that period. Morris (private letter), whilst not necessarily disagreeing with Piper's conclusion, believes that both the quality and quantity of palaeomagnetic data are insufficient to prove the case; and McGlynn *et al.* (*Nature*, **255**, 318; 1975) have produced data which seem to refute it. The problems in obtaining reliable palaeomagnetic directions, accurate ages and thus polar wandering paths from Precambrian rocks are well known. Nevertheless, the balance still seems to be in favour of a single supercontinent for at least the 2 billion years before the onset of Wegenerian drift

If this is confirmed it might have some significant implications. The environment represented by a single continent, whether or not surrounded by a spreading ocean floor, is rather different from that represented by a number of continents interspersed by spreading ocean floors. It is by no means certain therefore that plate-tectonic orogenic hypotheses derived from experience of the latter environment (which involves the interaction of rigid plates whose rigidity can be observed) would be directly applicable to the former. Equally, of course, it is by no means certain that such an extrapolation would not be possible. The point is that there is some reason to suspect that there could have been a change in the major causes of deformation with time, even though such a view could be held in some sense to weaken the strict uniformitarian doctrine. In practice, so strong has been the urge to accept uniformitarianism that there has often been a tendency not to debate the rights and wrongs of applying conventional plate-tectonic orogenic models to an alien environment but to change the environment to fit the hypothesis. Hence the invention of pre-Mesozoic intercontinental oceans—for example, the proto-Atlantic Ocean to explain the Palaeozoic Appalachian–Caledonian orogenic belt in terms of continent–continent collision. Presumably this sort of circular argument is partly why Le Pichon *et al.*, despite their previous statement, regard the attempt to explain older geological features in terms of plate tectonics as having "a certain dream-like quality".

The only person I know to have questioned uniformitarianism explicitly is Sutton (see, for example, Sutton, *Geol. Teaching,* **1**(1), 14; 1976, and Sutton and Watson, *Nature,* **247,** 433; 1974) who has modified the sacred doctrine with an element of evolution, chiefly the idea of a gradual increase in crustal rigidity with time. In Sutton's model the present phase of rigid plate activity, which began to develop about 1 billion years ago, was preceded by about 1.5 billion years of activity involving small permanent plates each a few hundred kilometres across. These small plates were aggregated into "a few large continental sheets which were themselves moving as essentially coherent units" and were separated within the sheets by mobile belts. Such a scheme obviously gives ample scope for the existence of intercontinental oceans; but if it were to be modified in terms of a single supercontinent, the interesting question would arise as to the extent to which the internal mobile belts would be flexible enough to allow internal spreading ocean floors to develop. For upon this might depend the validity of extrapolating current orogenic hypotheses to the pre-Mesozoic mountains which undoubtedly exist.

There is evidence both for and against pre-Mesozoic internal oceans. Taking all the 'favourable' evidence together, however, it may be in order to wonder whether the support it is said to give to ancient internal oceans may not also be based on circular reasoning. Broadly, the existence of ancient subduction zones is inferred from the recognition of rock types (such as mélanges and blueschists) characteristic of modern subduction zones But there is no reason in principle why different tectonic environments might not give rise to some similar rock types in different, though perhaps closely related, ways. Orogenic belts in two different environments may be due to causes similar enough to involve the formation of similar rocks, but not necessarily so alike that they need both involve subduction zones and plate collisions. To argue otherwise would surely be to adopt a form of uniformitarianism so strict that it would probably deny the possibility of two different tectonic environments in the first place. ☐

Genetic engineering

by Peter Newmark

A meeting on Genetic Engineering was held at the University of Glasgow on March 22–23, 1976. It was the 2nd Tenovus (Scotland) symposium of the Nucleotide Group of the Chemical and Biochemical Societies.

"GENETIC Engineering" is a fine title by which to attract the maximum attendance to a symposium. It is for some a sinister, for many an exciting prospect; it not only is seen by many molecular biologists as the path to their second coming but is also attracting considerable commercial interest.

Those who made the trip to Glasgow hoping for evidence of their wilder hopes or fears must have come away disappointed, for the meeting illustrated better the potential than the product of the techniques of cloning recombinant DNA.

The only real applications of cloning to be presented were in relation to the study of repetitive gene sequences. That topic was covered by D. D. Brown (Carnegie Institute, Baltimore), S. G. Clarkson (Zurich) and D. Glover (Imperial College, London). They have all been investigating the organisation of the hundreds or thousands of identical copies that exist for certain genes in eukaryotic chromosomes. The study of restriction enzyme fragments of repetitive genes has already made clear the basic organisation of their tandem repeats.

A finer analysis, however, of these naturally "cloned" genes has resulted from the experimental cloning of their restriction enzyme fragments. The availability of cloned fragments facilitates the preparation of heteroduplex DNAs for hybridisation and electron microscopic analysis by gene mappers. Clarkson has been able to place the *Xenopus laevis* gene for methionyl tRNA_{i} within a particular 1,900 base pair sequence. And Glover has discovered that the DNA sequence coding for 28S ribosomal RNA in *Drosophila melanogoster* is sometimes divided into two subunits separated by a non-transcribed spacer.

The most advanced dissection of repetitive gene sequences is that of Brown on the *X laevis* genes for 5S rRNA and 40S rRNA. In each case they are arranged as tandem repeats of a gene sequence and a non-transcribed spacer sequence. The transcribed sequences are all identical whereas the spacers show some variation in length. Their individual lengths are determined by the number of component small subunit sequences included in each spacer In somatic cell rDNA this heterogeneity in length has been localised to two distinct regions of the spacer sequence.

How is this spacer length variation distributed throughout the genome? The cloning of a tetramer fragment of a 5S rRNA repetitive gene has given Brown sufficient starting material to determine whether adjacent spacers have similar lengths They did not. It is therefore most unlikely that the evolution of the tandem genes involved anything like a rolling circle mechanism.

The soundness of that conclusion and of any others drawn from the use of cloned fragments will depend in part on the fidelity with which the initial fragment is copied during cloning H. M. Goodman (University of California, San Francisco) provided experimental assurance that assumptions of fidelity are, in fact, valid. He has cloned mouse mitochondrial DNA using a pSC101 plasmid vector in an *Escherichia coli* host and has compared the original DNA with that recovered after 25 generations of the host. On the basis of various criteria including restriction enzyme fragmentation patterns, electron microscopic contour length determinations and melting curve analysis, the cloned DNA was identical to its ancestor within the limit of detection of one base pair mismatch in 300.

There were two reports in addition to that of H. M Goodman of the cloning of mammalian genes. One was a very brief mention by K Murray (University of Edinburgh) of the cloning of mouse satellite DNA using a phage lambda vector; the other a full presentation by T. Rabbitts (MRC Laboratory of Molecular Biology, Cambridge) of his success in cloning a partial DNA copy of purified rabbit globin mRNA, using a mini-ColEI plasmid vector (see *Nature,* **260,** 221; 1976).

Gene cloning is clearly an established technique. The same cannot be said for the expression of cloned genes, an aim close to the heart of commercial genetic engineers who hope eventually to be able to harvest gene products such as interferon. But in "Will bacteria produce useful proteins from inserted eukaryotic genes?", N. H. Carey (Searle Research) was far from being able to answer his own question and J. Atkins (ICI) was not very much nearer. The latter is working with a phage lambda vector and a K. Murray host, a combination that at least has resulted in the successful translation of cloned prokaryotic genes, for example the production of penicillinase from the *Bacillus licheniformis* gene cloned in *E. coli.*

Another example of successful functioning, this time without translation of a cloned gene came from H M. Goodman. In a futuristic vein he presented evidence that it was possible to insert into the PM9 plasmid a chemically synthesised, double-stranded, 21 base pair DNA sequence of the *lac* operator. Transfection of *E. coli* with that recombinant DNA resulted in the constitutive production of β-galactosidase by some cells. The conclusion is that the plasmid, replicated to the extent of about 30 copies per cell, provided enough cloned copies of the *lac* operator to bind the cell's six repressor molecules.

Many of the speakers paid tribute to the technological contributions of E. Southern (University of Edinburgh) to their own work. E. Southern himself described both his "gene machine"—a high capacity, preparative agarose gel apparatus allowing 30-fold purification of restricton enzyme fragments—and his transfer technique. The latter technique involves the transfer of DNA fragments, banded in agarose gels, to cellulose nitrate filters on which they can be identified by hybridisation techniques.

Tribute should also be paid to N. M Wilkie (University of Glasgow) who told of his mapping of the herpes virus genome. By admitting his desire to clone herpes fragments he provoked a discussion on the hazards of cloning that had threatened never to surface. No amount of argument, however, could answer D. D. Brown's question of whether the cloned fragments of the herpes gene were any more hazardous than the infectious virus itself, and if not why prohibit the cloning? That particular problem is one of assessing the unpredictable and improbable hazards of cloned fragments against a known enemy. For the moment the fear of the unknown prevails. ☐

A hundred years ago

Mr. Tomlinson's remarks on safety-matches in Nature, vol. xiii, p. 469, reminded me that, not long ago I accidentally kindled one of those matches by rubbing it on the edge of a Wedgwood-ware mortar. This material appears even better adapted than those mentioned by Mr. Tomlinson for igniting such matches, and I found that a common earthenware dish (glazed inside) answered the same purpose admirably I tried to ascertain the degree of certainty with which a safety-match could be kindled by friction against these two materials, and was surprised to find that they are little inferior in this respect to amorphous phosphorus itself

from *Nature*, **13**, 512; April 27, 1876

Fossilised fury

from Barry Cox

Analyses of the mechanical adaptations of skeletons for movement and support, or of teeth for different kinds of diet, are comparatively simple. The interpretation of other structures, once used in the intraspecific social interactions of extinct animals, is far more difficult. Even in modern mammals, such a basic analysis as that of Geist on the evolution and use of horns is still only 10 years old. Where such living analogues are available, vertebrate palaeontologists are using these to explain some structures found in extinct animals, and are using similar techniques to explain other, more bizarre structures which find no obvious parallels in the world today.

In fact, Davitashvili's book *The Theory of Sexual Selection* (1961) included many suggestions as to the possible functions of unusual structures in fossil reptiles, but few non-Russian workers have read it. Some of his ideas have been independently put forward by other workers: for example, Galton (*J. Paleont*, **45**, 40; 1971) has suggested that the greatly thickened skull roof of the pachycephalosaurian dinosaurs was correlated with the use of the head in pushing and ramming during intraspecific combat, like that seen today in mountain sheep.

Geist himself suggested a similar function for the thickened heads of the herbivorous dinocephalian mammal-like reptiles, and Barghusen (*Paleobiology*, **1**, 295; 1975) has now convincingly shown that many other features of their skulls support this interpretation. Strong arches of bone transmit the impact force from the dorsal surface of the head to the occipital condyle. The line of action of this force also passes very close to this condyle, so reducing the torque that such blows would have produced at that joint. Barghusen also extends this explanation to the carnivorous dinocephalians, suggesting that, as in living suids, head butting and shoving allowed combat while reducing the danger of mutual injury from the massive incisor and canine teeth.

Geist (1966) clearly demonstrated that the primary use of the horns of many living herbivores is for intraspecific mating combat or the defence of territory, rather than for defence against predators. Farlow and Dodson (*Evolution*, **29**, 353; 1975) have now used this approach to analyse the horns and frills of the ceratopsian dinosaurs. They suggest that the most primitive ceratopsians, such as *Protoceratops*, used their small nasal horn for lateral blows against the flanks of their opponents, and used the bony frill that extended upwards and backwards from the skull as a visual dominance rank display structure, like the antlers of deer. Some later ceratopsians had a long frill and paired, diverging horns. Farlow and Dodson suggest that the frill would have been prominently displayed when these animals lowered their heads, and that the horns of rivals would have interlocked, leading to a trial of strength—again as in deer. Other ceratopsians, which had unpaired nasal horns and a short frill, they compare with rhinos, in which there is less display, the horns do not interlock, and injury is more frequent.

The strange cranial specialisations of the hadrosaurian dinosaurs have long been a fascinating problem, for they are unlike any structures found in living animals. In some, the bones of the nasal region form solid crests or humps, or are excavated around the nostrils; in others, these bones form elongated crests enclosing the nasal passages. Davitashvili originally suggested that the solid crests or lumps were combat structures and that the hollow elongate crests functioned as species recognition signals in courtship both visually and, by acting as resonating chambers, also audibly (as earlier suggested by Wiman).

Hopson (*Paleobiology*, **1**, 21; 1975) has now examined the implications of these ideas. He points out that if they are correct, various predictions should be fulfilled. For instance, as would be expected, the shape of the crest, being the result of independent selection for behavioural functions, is not merely a reflection of the size and shape of the internal cavity. The crests also appear to be dimorphic; otherwise identical skulls show variation in the size of the crest, and presumably belong to different sexes, while different sexes bear characteristically different crests. These species recognition features also, as would be predicted, are better developed in those faunas which contain a greater number of species of hadrosaur.

According to Hopson, the excavations around the nostrils of some hadrosaurs were filled with soft tissue, which could be inflated as a display structure and might also act as a resonator (as in elephant seals). He believes that the function of the elongate, hollow nasal tubes of other hadrosaurs was to provide a permanently dilated resonating system while at the same time providing a visual threat display.

The work of Geist and Davitashvili has provided a useful new approach for vertebrate palaeontologists. Though they cannot employ the experimental or observational methods in testing such hypotheses on fossil material, such techniques as Barghusen's analyses of associated structural adaptations, or Hopson's prediction-testing method, can provide adequate alternatives, and it is to be hoped that yet more fossil humps and bumps will find a function in the future. ☐

Liposomes to lysosomes

from a Correspondent

Now that many of the enzyme deficiences which cause the lysosomal storage diseases are known, efforts are increasingly being directed towards finding methods of therapy. One obvious approach is to replace the defective or absent enzyme (see for example, *Enzyme therapy in lysosomal storage diseases*, edit by Tager *et al.*, North Holland/American Elsevier, 1974). These diseases seem to be particularly suitable candidates for enzyme replacement therapy because extracellular macromolecules and particles are taken up by the process of endocytosis (phagocytosis) into the lysosomal system. Thus enzymes supplied (to the blood stream) may be expected automatically to reach the intracellular location where they are needed. They still, however, have to be directed to the particular tissue or tissues where most lysosomal storage occurs and these (brain and muscle, for example) often do not carry out much endocytosis. In order to remove the substances that have already accumulated and to maintain the corrected condition a regular supply of enzyme is required. Immunological complications arise from repeated injection of foreign antigens (either the enzyme itself or other substances copurified with it).

The idea of injecting enzyme entrapped in microvesicles, gel particles or red cell ghosts has recently been investigated A non-antigenic vesicle would not only prevent the enzyme from eliciting an immunological response but would protect it from degradation before it reaches the required site. Liposomes, which are synthetic vesicles made of concentric bilayers of lipids alternating with aqueous compartments in which the enzyme can be carried seem to be useful vehicles because they are non-antigenic and biodegradable and their dimensions and constituent lipids can be adjusted to suit requirements. Attempts are now being made to direct liposomes to target organs by coating them with substances which are known to have an affinity for and (or) be taken up by a particular tissue.

In a recent paper Cohen *et al.* (*Biochemistry*, **15**, 452; 1976) describe *in vitro* experiments on the entrappment of N-acetyl hexosaminidase A (the enzyme deficient in Tay-Sachs disease) by liposomes and the uptake of these liposomes by the polymorphonuclear leukocytes of a patient with Tay-Sachs disease. Selective endocytosis was encouraged by coating the liposomes with aggregated immunoglobulin (IgG), a successful idea based on the known uptake of immune complexes by polymorphonuclear leukocytes (Weissman *et al.*, *J. exp. Med.*, **134**, 149s; 1971). The liposomes were shown to be taken up by the lysosomes and N-acetyl hexosaminidase A was detected in the 'corrected' cells.

This type of *in vitro* experiment merits further study, not only to investigate the fate of the entrapped enzyme and the liposomal material, but also the breakdown of the accumulated substrate. The question of access of the liposome-entrapped enzyme to the 'residual bodies' of the lysosomal system where much of the stored material is found, needs to be resolved. Furthermore, as it is known that activity of N-acetyl hexosaminidase towards the natural substrate, the ganglioside GM_2 is lost on purification (Srivastava *et al.*, *J biol. Chem.*, **249**, 2043; 1974) it will be of great interest to see whether activity towards the natural substrate can be restored by replacing the purified enzyme within the cell.

While few animal models are available for the study of these diseases, *in vitro* experiments of this kind are clearly useful model systems. At the same time an investigation into the safety of the injected ingredients is of paramount importance and this can be done most conveniently using experimental animals. The release of lysosomal acid hydrolases which seems to occur parallel with the endocytotic uptake is a side effect that should be considered further. It is of interest to note that some of the pharmacological effects of liposomes are currently being investigated (Bruni *et al*, *Nature*, **260**, 333; 1976).

Successful treatment of Tay-Sachs disease as well as other infantile diseases with neurological involvement seems, however, unlikely to be practicable. The difficulty of penetrating the blood-brain barrier, a prerequisite for removing accumulated substrate from the brain is problem enough. Also substantial accumulation and possibly irreversible damage have already occurred as early as 16 weeks gestation (Schneck *et al.*, *Pediatrics*, **49**, 342; 1972). Treatment of juvenile and adult onset diseases (such as Fabry's disease) seems on the other hand to be a more hopeful and justifiable proposition. ☐

Triton interactions

from P. E. Hodgson

THERE have been rather few studies of the interaction of tritons with nuclei, mainly because tritons are highly radioactive, with a half-life of about 12 years. Very stringent safety precautions have to be enforced, so work with tritons is usually done in Government laboratories. A series of measurements of the differential cross sections for the elastic scattering of tritons by nuclei was made some years ago at Aldermaston, and analysed to give triton optical model potentials. There were hardly any measurements of triton polarisation and these were of low accuracy, so very little was known about the spin-orbit term in the triton optical potential.

Recently a polarised-triton source has been installed on the Los Alamos Scientific Laboratory Tandem Van de Graaff accelerator, and this has made possible new measurements of triton elastic scattering of a higher accuracy than ever before. The Los Alamos group have measured the differential cross sections and polarisations for tritons elastically scattered from several nuclei, and analysed the results with the optical model (*Phys. Rev. Lett.*, **35**, 1623, 1975).

It is possible to calculate the triton optical potential by averaging the known nucleon–nucleus interaction over the three nucleons comprising the triton. This folding model, as it is called, has already been used successfully to calculate the optical potentials for deuterons, helions, and alpha particles, so it is important to test it for tritons as well.

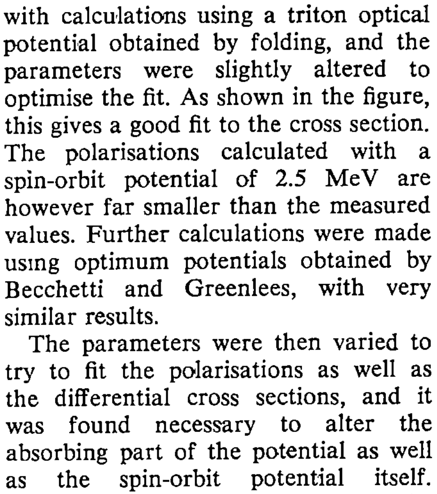
The folding model gives for tritons an optical potential that is about three times as deep as the nucleon optical potential, has about the same radius and is slightly more diffuse in the surface region. The two neutrons in the triton are anti-aligned, so that the spin-orbit force between a triton and a nucleus is the same as that between a nucleon and a nucleus.

Now the spin-orbit force is written as $V_{so}(r)\mathbf{L}\sigma$, where σ is the spin of the projectile and **L** the relative angular momentum between the projectile and the target. For the same incident velocity this angular momentum is three times greater for a triton than for a nucleon, so we expect the spin-orbit potential depth to be one-third the nucleon value to leave the whole potential the same. Since the depth of the nucleon spin-orbit potential for nucleons is about 6 MeV we expect a value of about 2 MeV for the triton spin-orbit potential.

To test this prediction, the differential cross sections were first compared with calculations using a triton optical potential obtained by folding, and the parameters were slightly altered to optimise the fit. As shown in the figure, this gives a good fit to the cross section. The polarisations calculated with a spin-orbit potential of 2.5 MeV are however far smaller than the measured values. Further calculations were made using optimum potentials obtained by Becchetti and Greenlees, with very similar results.

The parameters were then varied to try to fit the polarisations as well as the differential cross sections, and it was found necessary to alter the absorbing part of the potential as well as the spin-orbit potential itself. Potentials were found that fit the polarisations very well, but they have spin-orbit depths of 6 MeV, which is much greater than that given by the folding models.

The new data can therefore be successfully analysed by the optical model, but the strength of the spin-orbit potential is unexpectedly high, and poses a challenging theoretical problem. □



Polarisations (top) and differential cross-sections (bottom) for the elastic scattering of 15 MeV tritons by ^{60}Ni and ^{90}Zr compared with optical model calculations with a folding model potential (dashed lines) and with a Becchetti–Greenlees potential (full lines). Both potentials fit the differential cross-sections quite well, but give polarisations that are far too small.

Disturbed habitats

from Peter D. Moore

ONE of the basic aims of ecological research is the generation of theoretical models on the basis of which predictions can be made. The complexity of interactions in any ecological system make this a difficult task, but it is a particularly necessary one especially where a basis is required for the formulation of an informed management programme for a given habitat. As a consequence of this need, there has been a noticeable swing in ecological research in recent years away from the traditional study of 'natural' ecosystems to an unashamed interest in those habitats which have been subjected to some disturbance or perturbation at the hands, or feet, of man.

A good example is that of sand dune habitats, where the bulk of ecological research interest is no longer focused on the general course of successional process, but has become concentrated on the effects of trampling upon soils and vegetation. Some recent work in this field by Liddle and Grieg-Smith (*J. appl. Ecol.*, **12**, 893 and 909; 1975) has involved both descriptive and experimental studies on the Abeffraw sand dunes in Anglesey.

Examination of soils revealed that several changes occur with trampling. Trampled soils were found to have increased in their bulk density and, as might be predicted, the effect of a car was considerably more severe than the passage of a human being, the density being increased by a factor of about 30% more after a car has passed than after a man. Soil compaction was associated with higher water content, which could favour certain species sensitive to water stress in this arid environment, but the anaerobic nature of such soils would not encourage extensive root growth by most species.

Examination of the vegetation from transects across paths showed that although species number decreased towards the path centre, diversity remained fairly constant This suggests that loss in richness is accompanied by an increase in evenness of apportionment of biomass between species; this was probably caused by reduction in dominance with increased trampling pressure. Other observations show a fall in total biomass and an increase in the proportion of that biomass belonging to monocotyledenous species as trampling increases.

Unfortunately the vegetation data suffer from the disadvantage of having been gathered in June, when most of the winter annual species (mainly dicotyledons) would have already flowered and died. Since many of these species flourish under disturbed conditions, especially if sand is laid bare, they are likely to be an important winter and spring component of the path flora. Liddle and Grieg-Smith suggest that monocot : dicot ratio could prove a simple and useful index of trampling intensity, but seasonal factors need to be considered since there will be a greater proportion of dicots in winter.

Liddle (*Biol. Conserv.*, **8**, 251; 1975) has attempted to generalise yet further concerning the vulnerability of vegetation to trampling, on the basis of his sand dune data and further information derived from other habitats. He demonstrates a linear relationship between the logarithm of the number of people needed to pass along a linear path through a given vegetation type in order to reduce its biomass or cover by half, and the logarithm of the recorded primary productivity of that vegetation type. This relationship is not surprising, since more productive vegetation may be expected to regrow after trampling and so recover its former biomass more quickly. Liddle also considers that mature ecosystems are more resistant to trampling than early successional ones because they are more productive. This is not consistent with his own data, however, which show that short turf grassland containing such species as *Festuca rubra*, *Bellis perennis*, and *Poa pratensis* are particularly resistant to trampling yet are not mature in the successional sense, unless one regards the path community as a 'trampling climax', in which case the argument becomes a semantic one. The productivity/trampling resistance relationship may be genuine, but there is a danger of confusing net and gross ecosystem production when making successional inferences. Net ecosystem production does not rise during the early stages of succession, but then falls to zero at climax. Some of the most productive plant species are in fact found in immature ecosystems. It is therefore unwise to state that mature ecosystems are more productive, and therefore, more resistant to trampling.

The development of predictive models, or even the derivation of simple indices for the monitoring of man-induced changes is still some way off. But if Liddle's ideas on productivity are sufficiently compacted to hold water then we should be able to increase an ecosystem's tolerance to trampling simply by fertilising it. □

articles

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Variability of AO 0235+164 at radio, optical, and infrared wavelengths

The most recent outburst of the BL Lacertae object AO 0235+164, in November 1975, was particularly large and built up very quickly. These three papers present measurements made at radio, optical and infrared wavelengths, and discuss the extent to which the emitting regions in each case are associated.

Variability of AO 0235 + 164 at 2.8 cm.

THE radio source AO 0235+164 was discovered by Hazard and Sutton during lunar occultation observations at the Arecibo Observatory[1]. Spinrad and Smith[2] classified the optical object as of the BL Lacertae type. It is a moderately bright variable stellar object with an off-centre nebular extension to the south. In this issue of *Nature* Rieke *et al.*[3] report extensive optical observations which demonstrate the extraordinary nature of this object. The recent behaviour of the source at radio wavelengths is described here and in an accompanying paper by Ledden *et al.*[4].

AO 0235+164 was added to the list of sources observed regularly in the variable source programme at the Algonquin Radio Observatory[5] (ARO) in October 1972. Since then its flux density has been measured about once a month. The results are given in Fig. 1 and Table 1. The methods and errors of observation have been discussed by Medd *et al.*[5].

There have been at least three major outbursts in three years, the last of which, peaking in November 1975, had an extremely large and rapid increase. The ratio of maximum (7.6 Jy) to minimum (0.6 Jy) flux density observed for this source is ~ 12. This ratio is by far the largest we have observed for any source at 2.8 cm, the next largest being ~ 6 for BL Lac.

Fig. 1 The flux density of AO 0235+164 at 2.8 cm since November 1972.

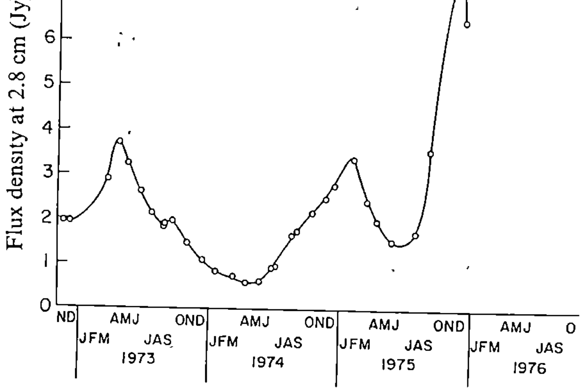


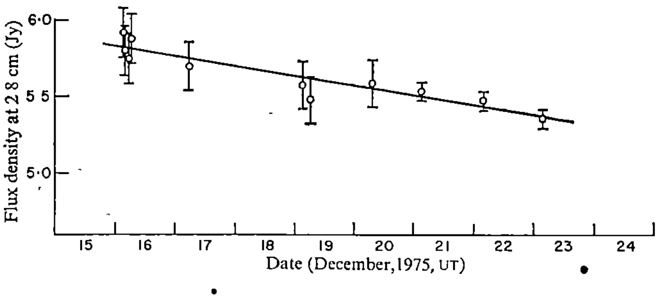
Table 1 Flux densities (Jy) of AO 0235+164 at 2.8 cm

Date	$S_{2.8}$	Date	$S_{2.8}$
1972		October 17	2.13
November 18	1.99	November 28	2.49
December 6	1.97	December 13	2.78
1973		1975	
March 22	2.89	February 6	3.38
April 19	3.73	March 16	2.41
May 8	3.23	April 12	2.00
June 22	2.62	May 27	1.53
July 22	2.13	July 30	1.74
August 24	1.84	July 31	1.71
August 27	1.90	September 3	3.58
September 14	1.96	September 4	3.51
September 15	1.97	November 19	7.43
October 31	1.47	November 25	7.67
December 11	1.09	December 7	6.47
December 13	1.07	December 16	5.92
			5.80
1974			5.75
January 18	0.85		5.88
March 4	0.74	December 17	5.70
April 12	0.60	December 19	5.58
May 23	0.64		5.48
June 25	0.95	December 20	5.59
July 4	0.98		5.54
August 22	1.66	December 21	5.48
September 2	1.75	December 22	5.36
October 15	2.20		

Such behaviour seems typical of BL Lac objects. The majority of other BL Lac objects in the ARO programme also show rapid variations (time scales $\lesssim 1$ yr) and $S_{\max}/S_{\min}$ ratios in excess of 2 (for example, PKS0048−09, DA55, OJ287, OT081, BL Lac) though a few do not (PKS0735+17, PKS1514−24) (B. H. Andrew, G. A. Harvey and W. J. Medd, in preparation).

Measurements of the flux density of AO 0235+164 during the period December 16–23, 1975 (Fig. 2) showed no evidence of daily variations > 0.1 Jy, though variations of 1 or 2 Jy have already been detected on this time scale in both OJ 287 (ref. 6) and BL Lac (ref. 7) on several occasions.

Fig. 2 The flux density of AO 0235+164 at 2.8 cm from December 16–23, 1975. There is no evidence for rapid variations superimposed on the gradual decay of the event of November 1975.



The Algonquin Radio Observatory is operated by the NRC of Canada. We thank Dr A. E. Neill for drawing this source to our attention, Drs P. C. Gregory and W. H. McCutcheon for making several of the observations in December 1975, and Dr P. A. Feldman for an observation in November 1975.

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Received January 30, accepted March 18, 1976

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Radio spectrum of the major outburst in the BL Lacertae object AO 0235+164

THIS paper presents an analysis of the evolution of the radio emission of AO 0235+164 during the recent large outburst which coincided with the optical and infrared event reported by Kinman and Rieke[1]. The event seems to be the clearest case to date of a correlated radio and optical outburst. Spinrad and Smith[2] suggested that AO 0235+164 is a red BL Lacertae object and noted that it is a radio and optical variable. Two smaller radio outbursts have been observed since 1972.8 by MacLeod *et al.*[13] and, based on their data and that of J. C. Webber (private communication), the quiescent flux density level of the source at frequencies > 1 GHz is < 1 Jy (1×10^{-26} W m^{-2} Hz^{-1}). The recent event described here offers an unusual opportunity to study an isolated outburst because its amplitude was much larger than the quiescent flux level, good coverage in time at two radio frequencies was obtained, and simultaneous observations over a wide range of radio, infrared, and optical frequencies were made.

The radio flux density data are presented in Tables 1 and 2. The measurement at 2.7 GHz was made with the 300-foot antenna of the National Radio Astronomy Observatory. Observations of flux density and linear polarisation at 4.9, 8.0 and 14.5 GHz were made with the University of Michigan 85-foot paraboloid using a previously described technique[3]. The measurements at 31.4 and 85.2 GHz were made with the 36-foot antenna of the NRAO as part of a millimetre wave variable-source programme being conducted by Hobbs and Dent. Included in Table 2 are a flux density measurement at 5 GHz made with the NRAO 300-foot antenna by J. W. Warner and J. H. Black (private communication) and the average of observations on two days at 90 GHz by B. J. Wills[4].

The characteristics of the observed radio radiation are consistent with the synchrotron radiation process. The emission extended over a broad range of frequencies, and the source was linearly polarised during the outburst by up to 3% at both 8 and

Fig. 1 The flux density against UT date at 14.5 (×) and 8.0 (○) GHz. The arrows labelled *a*, *b*, *c*, *d* indicate the epochs of the spectra shown in Fig. 2.

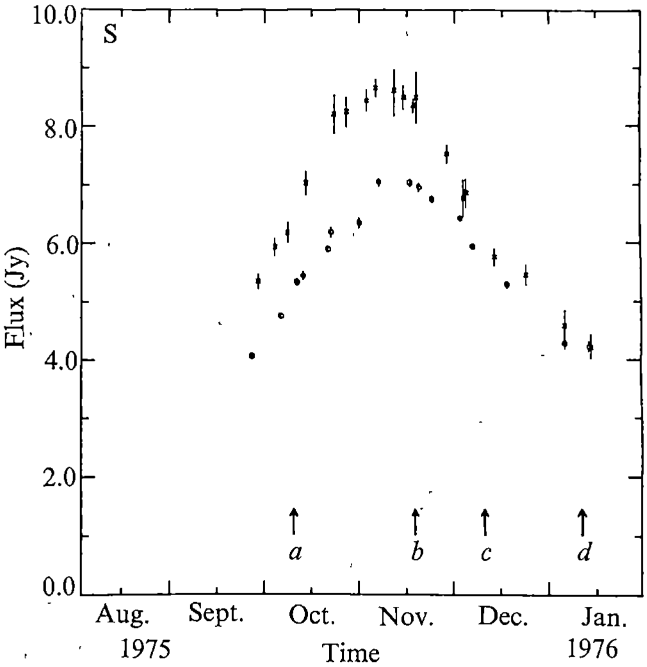

Table 1 Flux density variations with time*

Date (UT)	8.0 GHz S_ν (Jy)	8.0 GHz σ_S (Jy)	14.5 GHz S_ν (Jy)	14.5 GHz σ_S (Jy)
August 1974				
19	1.21	0.09		
September 1975				
27	4.05	0.06		
29			5.34	0.13
October 1975				
4			5.93	0.16
6	4.74	0.05		
8			6.17	0.18
11	5.33	0.07		
13	5.44	0.08		
14			7.02	0.20
15			7.21	0.59
21	5.89	0.06		
22	6.18	0.09		
23			8.20	0.34
27			8.24	0.26
31	6.34	0.10		
November 1975				
3			8.43	0.19
6			8.64	0.16
7	7.04	0.08		
November 1975				
12			8.60	0.45
15			8.48	0.21
17	7.02	0.08		
18			8.35	0.13
19			8.48	0.45
20	6.95	0.08		
24	6.74	0.07		
29			7.52	0.16
December 1975				
3	6.41	0.06		
4			6.75	0.32
5			6.84	0.25
7	5.93	0.05		
14			5.75	0.15
18	5.28	0.06		
19			6.31	0.50
24			5.45	0.18
January 1976				
6	4.26	0.09	4.57	0.27
13	4.23	0.10	4.21	0.21

* Observations made with University of Michigan 85-foot antenna.

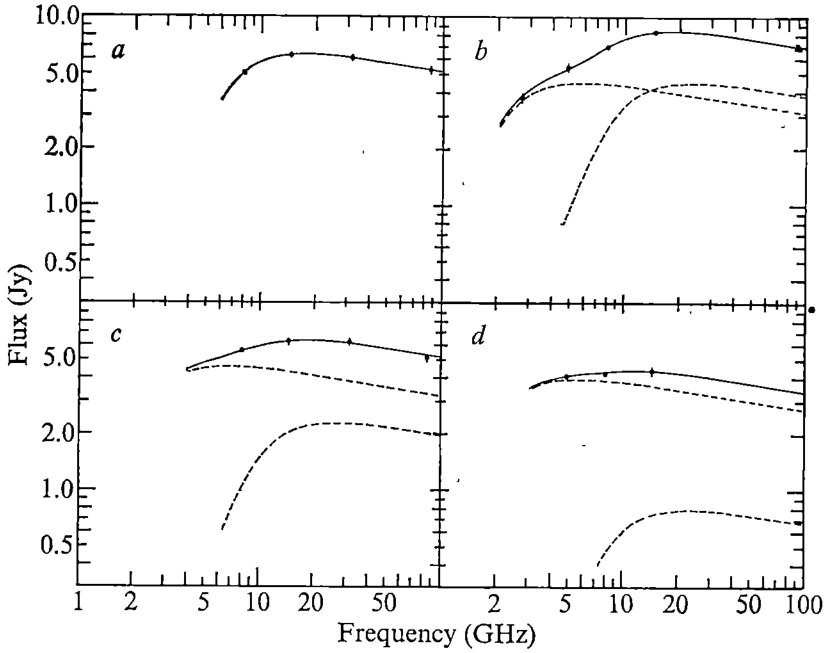

Fig. 2 Spectrum of AO 0235+164 at four epochs: *a*, October 9, 1975; *b*, November 18, 1975; *c*, December 10, 1975; and *d*, January 10, 1976. The solid curves were fitted to the data by summing the two self-absorbed homogeneous synchrotron source spectra shown as dashed lines. The values shown at 8.0 and 14.5 GHz are interpolations of the data shown in Fig. 1. In *b* the 5-GHz data point is an average of two measurements made on November 4 and November 24, 1975 and the 90-GHz data point is an average of measurements made on November 20 and November 21, 1975.

14.5 GHz and to a higher degree at optical frequencies[4]. A preliminary analysis of our linear-polarisation data indicates no significant difference between the observed polarisation position angles at 8 and 14.5 GHz, which is consistent with the hypothesis that there is no Faraday rotation within the source. The polarisation position angle at 14.5 GHz during early October 1975 was 162±8° which agrees, within our error, with the polarisation position angle observed at optical wavelengths by Kinman[4] on October 3, 1975. This agreement certainly suggests that the radio and optical radiation originated in regions having the same magnetic-field orientations, but the much lower degree of polarisation at 14.5 GHz (2.2±0.6%) indicates that the magnetic field in the radio-emitting region is less well ordered. These polarisation data are evidence of a direct association between the optical and radio-emitting regions.

The time dependence of the radio outburst at 8.0 and 14.5 GHz is shown in Fig. 1. Before the maximum in mid November the fractional rate of increase was the same ($1.7\%\,d^{-1}$) at the two frequencies. The maximum at 14.5 GHz seemed to occur on November 8 which was ~ 6 d before the apparent maximum at 8.0 GHz. Such a delay is qualitatively consistent with expanding synchrotron-source models, but the observed ratio of 1.2 for the flux densities at maxima is significantly less than the ratio of 2.0 predicted by the instantaneous injection model[5]. The observed behaviour of the flux density at the two frequencies could be explained by either a prolonged injection or an acceleration of radiating particles from August through mid November 1975 (ref. 6). The complex light curve at infrared and optical wavelengths also reached a maximum in mid November[4] and appears to be direct evidence of just such a prolonged injection.

Table 2 Spectral data

Date (UT)	Frequency (GHz)	S_ν (Jy)	σ_s (Jy)	Antenna
October 9, 1975	31.4	6.09	0.21	36′ NRAO
	85	5.21	0.27	36′ NRAO
November 5, 1975	4.9	5.47	0.32	85′ Univ. Michigan
November 18, 1975	2.7	3.74	0.20	300′ NRAO
November 20.5, 1975	90	6.97	0.20	36′ NRAO
November 24, 1975	5.0	5.70	0.30	300′ NRAO
December 10, 1975	31.4	6.24	0.18	36′ NRAO
	85	5.04	0.10	36′ NRAO
January 10, 1975	4.9	4.10	0.15	85′ Univ. Michigan

The observed radio spectra of the source at four epochs are displayed in Fig. 2. The most striking feature of three of the spectra is the broad low frequency turnover which cannot be fitted by a single homogeneous, self-absorbed synchrotron emitting region but which is characteristic of inhomogeneous synchrotron sources[7]. As the simplest representation of the spectrum of an inhomogeneous source, we have summed the spectra (shown as dashed lines) of two homogeneous emitting regions which become self-absorbed at different frequencies. The progressive flattening of the spectrum at <14.5 GHz after the maximum in mid November (see Fig. 1) is consistent with the turnover progressing towards lower frequencies. This phenomenon is illustrated by the systematic decrease in the amplitude of the high frequency spectral component in Figs 2*c* and 2*d*. Above 30 GHz the source was completely transparent during the period of observation, and our data are consistent with the spectral index α remaining constant at $\alpha = -0.15$. The fact that the source was transparent before reaching maximum is inconsistent with instantaneous-injection expanding source models. It requires that the injection or acceleration of the radiating particles was prolonged and that energy was supplied to the radiating particles more rapidly than it was lost due to expansion of the emitting region. Synchrotron and inverse Compton energy losses seem to have been unimportant for particles radiating at < 100 GHz, as there was no significant steepening of the spectrum with time.

An extrapolation of the radio spectrum in mid November predicts a $\lambda = 20$ μm flux density of 3 Jy compared with an observed flux of 1 Jy (ref. 4). The continuity between the radio and infrared fluxes suggests that the emission over the entire spectrum is produced by synchrotron radiation. The observed spectral index of -1.2 in the infrared and -3.8 at optical wavelengths[2,4] indicates that the synchrotron emission cuts off in the latter spectral region.

A quantitative analysis of the observed emission places additional constraints on models for the source because of the apparent high brightness temperature implied by the rapidity of the variations. Based on the observed fractional rate of change of the flux density, the apparent radius of the source must be $\lesssim 100$ light d. Two absorption line redshift systems, with $z = 0.524$ and $z = 0.851$, have been observed in the optical spectrum[4]. The larger redshift would correspond to a cosmological distance of $\sim 6\times10^9$ pc. This distance and apparent physical size yields an angular diameter of 2×10^{-5} arc s and implies an apparent source brightness temperature of $T_b \simeq 10^{15}$ K at 8 GHz. Such a high intensity would result in the inverse Compton process[8,9] producing a much larger flux of infrared and shorter wavelength radiation than was observed. The observed maximum flux density in the optical B band of 3.7×10^{-3} Jy (ref. 4) can be used to set an upper limit on the radio brightness temperature[10] of $\sim10^{12}$ K at 8.0 GHz. The disparity between this upper limit and the apparent observed brightness temperature is eliminated if the source expands with a relativistic velocity[10,11] or if it is much closer than the cosmological distances associated with the observed redshifts. An expansion velocity corresponding to a Lorentz factor of ~ 10 is sufficient to reduce the brightness temperature in the reference frame of the emitting region to $< 10^{12}$ K and would make the inverse Compton process energetically unimportant in the source. A relativistic expansion of the source would also produce a broadening in the low-frequency self absorption turnover of the spectrum[12] which is qualitatively consistent with our observed radio spectra of AO 0235+164.

We thank R. W. Hobbs, T. J. Balonek, J. H. Black, J. W. Warner and J. C. Webber for allowing us to use their unpublished data; F. T. Haddock, T. D. Kinman, G. H. Rieke and B. J. Wills for discussions; T. V. Seling, the staff of the University of Michigan RAO and the staff of the NRAO for making these observations possible. The NRAO is operated by Associated Universities, Inc., under contract with the NSF. This research was supported in part by grants from the NSF.

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Received February 2, accepted February 25, 1976.

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Photometric and spectroscopic observations of the BL Lacertae object AO 0235+164

BL LACERTAE objects pose, in extreme form, the question of the nature of the most energetic extragalactic sources. They exhibit rapid variability over a large dynamic range, large and variable intrinsic polarisation, and high luminosity, a combination that is very difficult to reconcile with current physical theory. In this paper, we confirm that AO 0235+164 is a BL Lacertae object with one absorption-line redshift at $z = 0.524$ and a probable one at $z = 0.852$. Photometry between 0.36 and 21 μm and at 90 and 140 GHz showed a peak luminosity in November 1975 comparable with the most luminous QSOs.

Extensive studies in the optical and radio spectral regions have documented the variations in flux and polarisation for a number of these sources[1,2]. The available infrared observations are far less complete, even though BL Lac-type objects emit a large, frequently dominant, portion of their energy in this spectral region. Coordinated programmes to study the behaviour of these sources in all three spectral regions are needed.

Their lack of emission lines has made it difficult even to estimate the distances of these sources. Two absorption lines identified as the Mg II doublet redshifted by $z = 0.424$ have, however, been found in the spectrum of one of these objects (PKS0735+178) (ref. 3). In addition, BL Lac itself and some probably similar objects (1101+38, 1652+39; ref. 2) have surrounding nebulosity which shows absorption lines at redshifts of $z < 0.1$.

AO 0235+164 is a newly discovered member of this class of object. Based on the coincidence of precise optical and radio positions, it was identified by Argue, Kenworthy, and Stewart[4] with a 19-mag, apparently stellar object. Spinrad and Smith[5] found that the optical object had a featureless spectrum at wavelengths > 4,400 Å and varied between $V = 17.5$ and 19.5 during 1972–74. They reported that the object was red ($B\text{–}V \simeq 1.3$) and had an apparent faint wisp of nebulosity extending 3″ S from the stellar source. Spinrad and Smith also cited radio observations that show a spectrum rising with increasing frequency and indicate variability at 6 cm; they suggested that AO 0235+164 is a BL Lac-type object with an unusually red colour.

The outburst of AO 0235+164

We have studied the recent outburst of this source in which it reached a magnitude of $V = 14.3$. Its spectrum has a strong and rich absorption-line system at $z = 0.524$, and probably a second system at $z = 0.852$. If these redshifts are of cosmological origin, AO 0235+164 reached a peak luminosity $\geqslant 3\times10^{14} L_{\odot}$, comparable with the outputs of the most luminous QSOs. Most of this energy fell in the infrared. AO 0235+164 is one of the most rapid variables and has one of the largest amplitudes and linear polarisation in the optical region found for any QSO-like object. Extensive observations show that the shape of the infrared spectrum fluctuated during the outburst. Our radio observations supplement those reported in papers by MacLeod *et al.*[27] and Ledden *et al.*[6] which provide a thorough record of the outburst in this spectral region.

On October 3, 1975, UT, AO 0235+164 was 2 mag brighter than its brightest reported by Spinrad and Smith. On the following night, it had brightened by a further 0.18 mag. Further photoelectric photometry, obtained occasionally during the outburst, was supplemented by photographic observations of the *B* magnitude. All of these measurements are summarised in Table 1 where they have been converted to flux densities using the calibration of Johnson[7]. The linear polarisation of AO 0235+164, measured with an unfiltered S-11 photocathode at the Kitt Peak National Observatory (KPNO) 4-m telescope, was $24.6\pm0.5\%$ at a position angle of 154.2° on October 4, 1975, UT, and only $6.1\pm1.2\%$ at a position angle of 126.4° on January 4, 1976, UT.

Measurements

The large range and rapidity of the optical variations and the high and varying polarisation are characteristic of BL Lac-type objects and confirm the classification by Spinrad and Smith. It is noteworthy that the $B\text{–}V$ colour (0.98 mag) was about the same as that found by Spinrad and Smith before the source had brightened by more than a factor of 10. The *UBV* colours of AO 0235+164 are similar to those of weak-lined red degenerate stars and are slightly redder than those of weak-lined subdwarfs, emphasising the importance of precise optical and radio positions for identifying objects of this type.

Infrared measurements, also summarised in Table 1, were begun on October 7, 1975, UT. It was possible to observe the source approximately every two weeks during the outburst because of a fortuitous arrangement of telescope schedules. Near maximum, AO 0235+164 was sufficiently bright that complete infrared spectra extending from 1–21 μm could be obtained. The star α Ari was used as a flux standard and the photometry was reduced according to the calibration described by F. J. Low[28]. Where appropriate, the measurements

Table 1 Photometric data for AO 0235+164

µm	0.36	0.44	0.55	1.24	1.6	2.2	3.6	4.85	8.4	10.6	11	12.6	19	21
Waveband	*U*	*B*	*V*	*J*	*H*	*K*	*L*	*M*		*N*				*Q*
Date (UT)														
1975														
Oct. 3	0.40*	1.12	2.38											
Oct. 4	0.47*	1.34	2.81											
Oct. 7										144(20)‡				
Oct. 8		1.88(.18)†		30(3)‡		72	128							
Oct. 10		1.90(.16)§												
Oct. 11		1.97(.12)§												
Oct. 12		1.75(.15)§												
Oct. 26				26*	36.5	67	127	280(35)	290(50)		330(40)	370(70)		
Oct. 27	0.76″	2.01	4.03	27*	39	70								
Oct. 28	0.67″	1.77	3.44											
Nov. 8								160‡(50)		500				1,060(190)
Nov. 9				36‡	53	83	150	200(40)		525				660(120)
Nov. 11				48‡	66	108	182	240(25)						
Nov. 14		3.52(.37)§												
Nov. 24	0.57*	1.54	3.26											
Nov. 27				24*	29	53	105	230(55)	280(50)		300(40)		430(90)	
Dec. 1						65‡(10)				262(30)				650(260)
Dec. 2		1.94(.40)†		23‡(1.4)	37	59	108			200(25)				860(170)
Dec. 3		1.54(.11)†												
Dec. 18				14.6●	21.5	36	62							
Dec. 19						31″				237(40)●				
1976														
Jan. 3	0.11*	0.27	0.59											
Jan. 4		0.28*												

All flux densities are given in units of 0.001 Jy; when errors exceed 5%, they are given in parentheses.
*KPNO 4-m telescope.
†KPNO 0.3-m Schmidt telescope.
‡NASA 1.54-m telescope.
§KPNO 0.4-m telescope (Number 3).
″KPNO 2.1-m telescope.
●Steward Observatory 2.25-m telescope.

The photographic observations on the KPNO 0.3-m and 0.4-m telescopes were made by C. T. Mahaffey using Kodak IIa-o emulsion and a Schott GG-385 filter. The photoelectric magnitudes of the comparison stars that were used to calibrate these plates (together with their coordinates relative to AO 0235+164) are:
(A) $V = 15.00$; $B-V = 1.21$ (13″ E; 21″ N).
(B) $V = 13.04$; $B-V = 0.58$ (32″ W; 32″ S).
(C) $V = 16.77$; $B-V = 1.49$ (7″ W; 78″ N).

have been corrected for the effects of the bandpass filter on the apparent fluxes[8].

Discussion of photometry

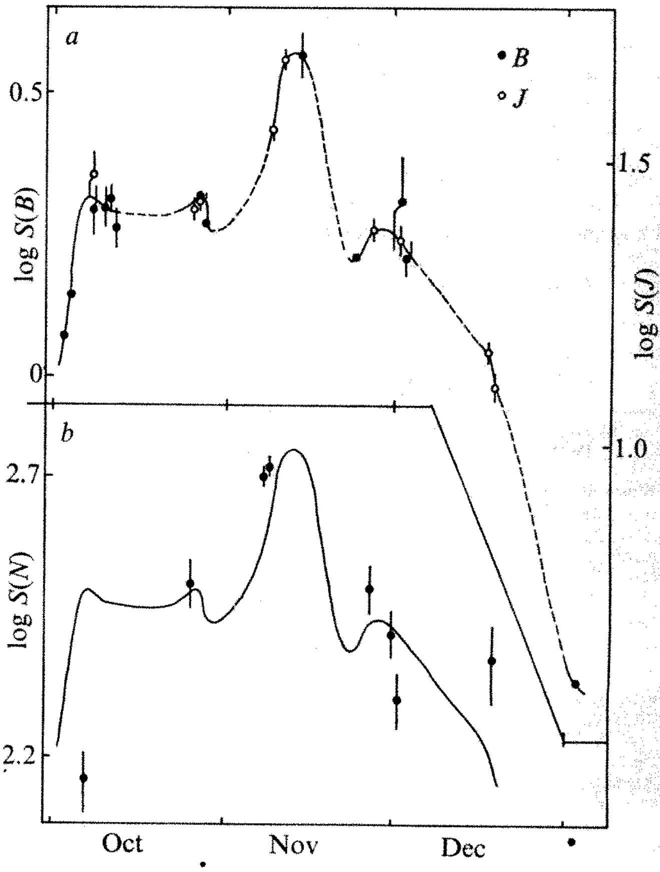
The infrared and visual light curves of AO 0235+164 are illustrated in Fig. 1. The behaviour of the source was roughly similar to that in a previous outburst during 1939–40, seen on plates in the collection of Harvard College Observatory[9]. The light curve is also similar to those of other BL Lac-type objects during violent outbursts, although AO 0235+164 is one of the most rapidly variable of this class.

From Fig. 1*a* it can be seen that the variations in the blue and near infrared are the same within the errors. The 10-µm observations, shown in Fig. 1*b*, give about the same amplitude as the visual curve, but appear to show different short time scale behaviour. The $U-B$ colour also varied slightly during the outburst. The radio flux reached a maximum at about the same time as in the optical and infrared, but the radio amplitude was considerably smaller[6]. From a comparison with the radio variations, because the source was relatively faint during our first observations, and because of the steep rise in the first part of the light curve, we believe that our first measurements were made during the initial phase of the outburst.

Variability of up to 30% on time scales of 1 or 2 d was seen both in the visible and infrared. Although we did not obtain sufficient simultaneous photometry in these two wavelength regions to prove that these fluctuations always occur simultaneously, that conclusion is indicated, since first, the infrared increase between November 9 and 11 was accompanied by an easily noticeable brightening as viewed through the telescope, and second, the spectral shape in each region implies that any variations should be simultaneous. Rapid variations of such large amplitude were not seen in the radio flux[6].

The photometric spectrum of AO 0235+164 on six occasions during the outburst is shown in Fig. 2. Where simultaneous measurements at all wavelengths were not available, the necessary extrapolations were based on Fig. 1*a*. The average correction for galactic extinction at the latitude of AO 0235+164 (−39°) is only 0.01 mag in $U-B$ and 0.02 mag in $B-V$

Fig. 1 Light curves of AO 0235+164 with flux densities in mJy. The upper panel (*a*) is for *B* (0.44 µm) and *J*(1.25 µm) except that the last point of *J* was obtained from a measurement at 2.2 µm and the mean [1.25 µm–2.2 µm] colour. The lower panel (*b*) is for *N*(10 µm); some narrow-band measurements near this wavelength have been combined to increase the statistical weight. The curve is the same as that used in (*a*).



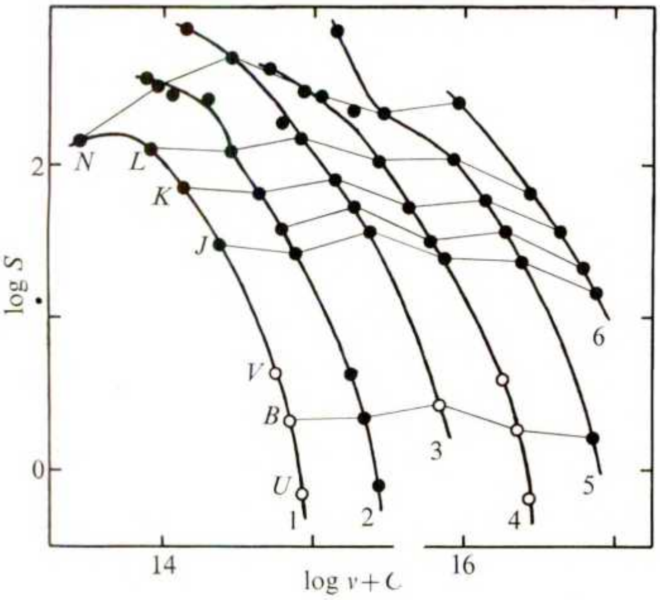


Fig. 2 Photometric spectra of AO 0235+164 with flux densities in mJy. Thick lines join measurements taken at the same time while thin lines connect measurements at the same frequency. For clarity, successive epochs are displaced sideways. The 1975 UT dates and values of the constant C are: curve 1—Oct. 7–8, $C = 0$; curve 2—Oct. 26–27, $C = 0.5$; curve 3—Nov. 8–9, $C = 1.0$; curve 4—Nov. 27, $C = 1.5$; curve 5—Dec. 1–2, $C = 2.0$; curve 6—Dec. 18–19, $C = 2.5$. ○, points that were extrapolated according to Fig. 1*a* by > 24 h.

(ref. 10), and there is no indication of strong localised extinction near the source. The (10.6–0.44 µm) and (0.55–0.36 µm) spectral indices varied over the ranges -1.49 ± 0.14 and -4.14 ± 0.12, respectively. Although the spectra of most BL Lac-type objects are slightly curved between 0.4 and 10 µm (ref. 11), that of AO 0235+164 has substantially greater curvature and is steeper than is typical.

The spectra in Fig. 2 show the effects of the different short term variability beyond 4 µm. Although these effects are apparent throughout the outburst, they are particularly striking at the beginning when the spectrum appears to turn over near 10 µm. Another explanation — that the source brightened by a factor $\gtrsim 2$ between October 7 (when the 10 µm data were obtained) and October 8 (when the other measurements were made)—seems unlikely because there was no noticeable brightening observed visually between these two dates, and fluctuations of the required amplitude and speed were not seen at any other time. Instead, it seems that the flux rose more slowly at 10 µm than at shorter wavelengths.

The 90 and 140 GHz measurements of AO 0235+164, summarised in Table 2, together with other mm-wave flux densities, indicate a maximum which occurred at about the same time as in the optical and infrared, but they show a slower decay. The radio outburst is discussed in detail in refs 6 and 27.

Table 2 High frequency radio observations of AO 0235+164

Date (UT) 1975	Flux density (Jy)	Frequency (GHz)	Telescope*
Nov. 20	6.9 ± 0.3	90	A
Nov. 21	7.2 ± 0.3	90	A
Nov. 22	6.6 ± 0.3	90	A
Nov. 23	7.0 ± 0.3	90	A
Nov. 28	6.1 ± 1.2	140	B†
Dec. 1	3.4 ± 1.4	140	B†
Dec. 18‡	5.4 ± 0.4	90	A

*A = 11-m telescope of the National Radio Astronomy Observatory at KPNO which is operated by Associated Universities, Inc., under contract with the National Science Foundation.

B = 4.9-m telescope of the mm-Observatory operated by the Electrical Engineering Laboratory of the University of Texas at Austin, with support from NASA, NSF and the McDonald Observatory.

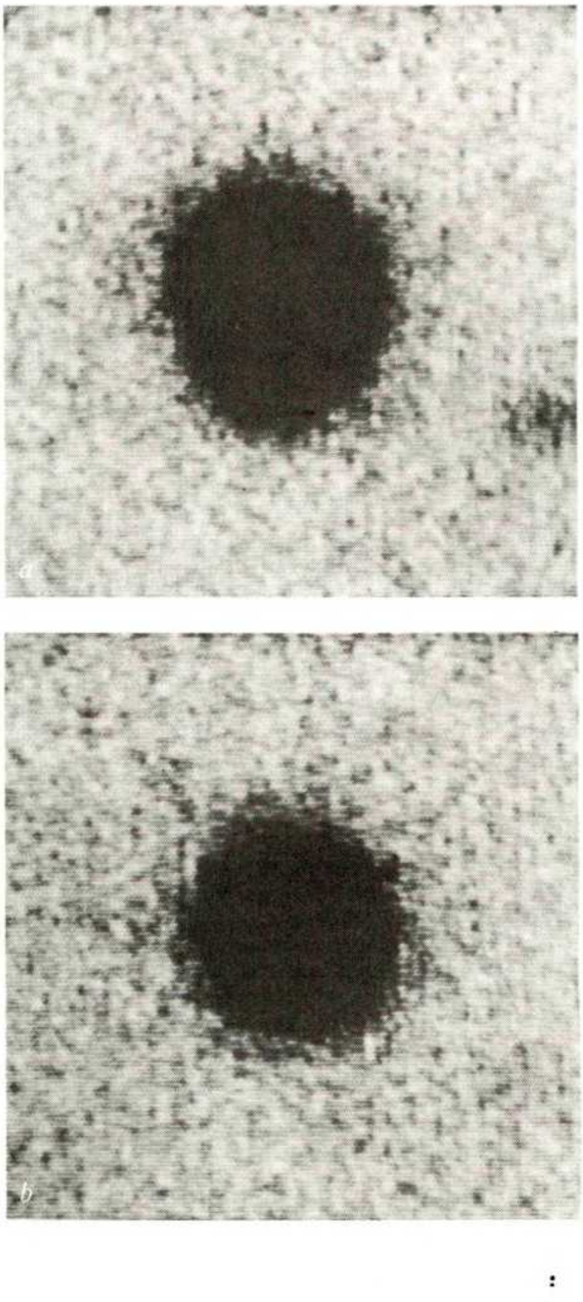
†These observations were made with assistance from A. Uomoto.

‡This result was kindly made available by Dr E. K. Conklin.

Photographic results

A deep plate in the yellow–red (Eastman Kodak 127-04 emulsion and Schott GG-495 filter) was taken at the prime focus of the KPNO 4-m telescope on January 4, 1976, UT, when the object was at $B \simeq 18.0$. The image of AO 0235+164 on this plate, and that of a star of comparable brightness (6″W and 187″N), were scanned on the PDS microphotometer at KPNO using a square raster scan of side 13″ and a scanning aperture of 0.15″. These data were displayed on the COMTAL television monitor of the KPNO Interactive Picture Analysis system[12]. Identical optimum adjustments were made to show the faint extensions of the images; photographs of this display

Fig. 3 Photographs of a television reconstruction of the images of *a*, AO 0235+164 and *b*, a nearby star to show the southerly extension of the former. North is to the top and E to the left, and each square has a side of 13″.



are shown in Fig. 3. The southerly extension of the image of AO 0235+164 found by Spinrad and Smith is confirmed, but its maximum extent (~ 3″) is no greater than that reported by them, even though the KPNO plate is deeper. A nebula or galaxy associated with the source is an exciting possibility, but our observation is also consistent with the existence of a faint, superposed star.

Absorption-line spectrum

A spectrogram of AO 0235+164, obtained under poor conditions on November 25 with the Steward Observatory 2.25-m telescope, showed an absorption feature which was identified as the Mg II doublet. Spectrograms were independently obtained between December 3 and 8 (UT) using a two-stage image-tube spectrograph on the 2.7-m telescope of McDonald Observatory. The reciprocal dispersion was 110 Å mm^{-1} and baked IIIa-J emulsion was used, giving a resolution of ~ 3 Å over the spectral range 3,500–5,500 Å. As an illustration, a density tracing of one of these spectrograms is shown in Fig. 4. Some of the features are clearer on other spectrograms; in particular, the lines near 3,600 Å are seen most clearly on a spectrogram optimised for that region. The existence of some of the weak lines is supported by their detection on a number of spectrograms.

The principal feature in the spectrum is a well resolved pair of deep, sharp absorption lines which we identify as the Mg II ($\lambda = 2,795.5$ and 2,802.7 Å) doublet at a redshift $z = 0.5242 \pm 0.0002$; the width of these lines is < 2 Å in the rest frame. There are many other absorption lines, identified as Mg I, Fe II, and Mn II; these are listed in Table 3, together with the number of spectrograms on which each line was measured.

All the lines listed as strong in Table 3 were also seen in PHL938 (ref. 13) and in PKS1331+170 (ref. 14) and are those predicted by Bahcall[15] to be the most easily detectable in QSOs within the range of rest-wavelengths covered by our spectrograms. The Mg I and Mn II lines have not been found previously in QSO spectra, but their identification is supported by a comparison with the ultraviolet spectrum of ζ Oph (ref. 16, and E. M. Burbidge, *et al.*, ref. 29). In fact, all the lines which occur in the appropriate wavelength region for the $z = 0.524$ system and which have equivalent widths > 30 mÅ in the spectrum of this star are also seen in the spectrum of AO 0235+164. The rich absorption-line spectrum at $z = 0.524$ removes any doubt about the correctness of the identification of the Mg II doublet in this object and, by analogy, in PKS0735+178.

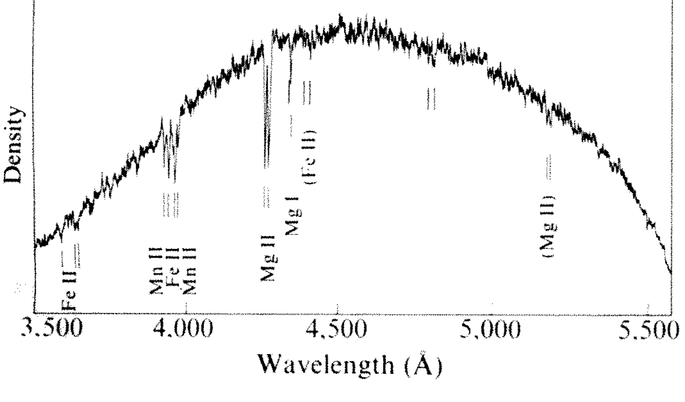
On three of the spectrograms there is a pair of absorption lines near 5,176 and 5,190 Å which cannot be readily identified if their redshift is 0.5242. Figure 5*a* shows the averaged density scan of these spectrograms; we suggest that the lines are the Mg II doublet at a redshift of 0.8516 ± 0.0007. Since these Mg II lines are only about one-quarter of their strength in the $z = 0.524$ system, even the strongest Fe II lines should be only barely detectable in the 0.852 system. A weak line measured at $4,412.2 \pm 0.7$ Å on three spectrograms corresponds to Fe II ($\lambda = 2,382$ Å) and the composite tracing shown in Fig. 5*b* lends further support to the reality of this line. The dip near 4,811 Å in Fig. 4 corresponds to the expected position of Fe II ($\lambda = 2,599$ Å) in the $z = 0.852$ system; it was not seen on the other three spectrograms, but they were less densely exposed in this wavelength region. The Fe II ($\lambda = 2,343$ Å) line would occur only 8 Å to the blue of the Mg I line (see Fig. 5*b*) and would therefore be difficult to detect. Since the redshifts of emission-line systems in QSOs are usually at least as large as the largest absorption-line redshift, the continuum of AO 0235+164 is probably at $z \geqslant 0.852$. Our spectroscopic results are in good agreement with those of Burbidge *et al.*[29], although our measurements extend further into the ultraviolet.

Table 3 Absorption lines in AO 0235+164

λ_{obs}	Strength*	Identification	λ_o	Redshift	No. of spectrograms measured
3572.1	S	Fe II	2343.5	0.5243	2
3599.2?	W				
3618.8	S	Fe II	2373.7	0.5245	2
3631.4	S	Fe II	2382.0	0.5245	2
3927.6	W	Mn II	2576.1	0.5246	3
3941.2	S	Fe II	2585.9	0.5241	3
3953.7	W	Mn II	2593.7	0.5243	2
3963.0	S	Fe II	2599.4	0.5246	3
3972.2	W	Mn II	2605.7	0.5244	3
4260.6	S	Mg II	2795.5	0.5241	3
4271.9	S	Mg II	2802.7	0.5242	3
4347.0	M	Mg I	2852.1	0.5241	3
4412.2	W	Fe II	2382.0	0.8523	3
4432.2?	W				
5176.0	W	Mg II	2795.5	0.8515	3
5189.7	W	Mg II	2802.7	0.8517	2

*S, M, W = strong, medium, weak absorption relative to the continuum.

Fig. 4 A density tracing of a spectrogram of AO 0235+164. Symbols without parentheses denote line identifications in the $z = 0.524$ redshift system, while symbols within parentheses indicate lines in the $z = 0.851$ system. The vertical lines without symbols show the expected positions that weaker members of the $z = 0.524$ system would have with a redshift of $z = 0.851$.



Emission lines

AO 0235+164 showed no emission lines on any of our spectrograms, including one exposed between 3,700 and 7,000 Å at a reciprocal dispersion of 240 Å mm^{-1}, which should have been particularly sensitive to broad lines. The presence of strong absorption lines without emission lines is very uncommon among known QSOs, a situation that may be partly a result of selection effects. Schmidt[17] noted the rare case of 4C 28.25, a QSO with an absorption-line system consisting of the Mg II doublet and the Fe II lines $\lambda = 2,382$ Å, $\lambda = 2,586$ Å, and $\lambda = 2,599$ Å with a redshift of $z = 0.8907$. 4C 28.25 also has weak emission features identified with C III and Mg II with a redshift of $z = 0.899$. 4C 28.25 is, therefore, intermediate between an emission-line QSO and objects like AO 0235+164. We also note that incipient emission with a redshift close to that of the absorption spectrum could distort the profiles of the absorption lines in AO 0235+164 and invalidate estimates of multiplet ratios.

Discussion

The relative line strengths in the $z = 0.524$ system may be difficult to reconcile with strictly solar abundance ratios in the gas and simple density and velocity distributions. The arguments in this paragraph are supported by the detection of structure in the 21-cm absorption line at $z = 0.52385$[30]. For example, explaining the relative strengths of the Mg II and Fe II lines by having them form lower on the curve of growth than in ζ Oph (E. M. Burbidge, *et al.*, ref. 29)—assuming a relatively

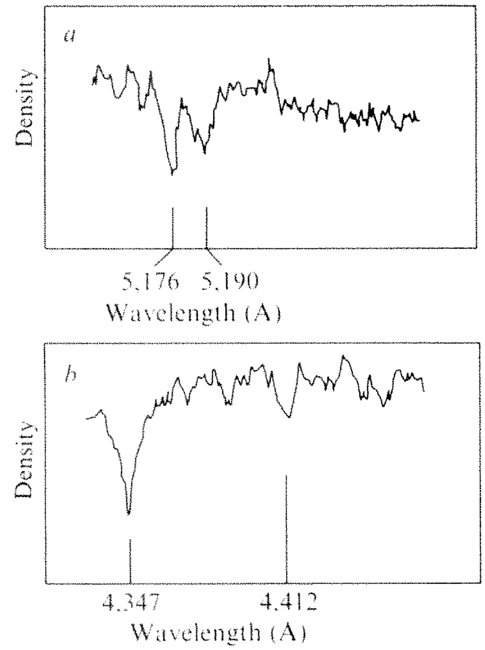


Fig. 5 Composite density tracings of the spectrograms to show the features in the $z = 0.852$ redshift system: *a*, the region of the Mg II doublet (5,176 and 5,190 Å) from three spectrograms and *b*, the region of the Fe II at $\lambda = 2{,}382$Å (4,412 Å) from four spectrograms.

low column density and high velocity dispersion—would require an overabundance of Mn and would be hard to reconcile with the relative strengths of the Fe II lines at 3,619, 3,631, 3,941, and 3,963 Å. If the relative strengths of the Mg II and Fe II lines are a result of a very high column density and small velocity dispersion[18], the Fe II lines should be more strongly saturated than indicated by the equivalent widths given by Burbidge *et al.*

High column densities might also result in heavy extinction, particularly if the absorption lines arise in an intervening galaxy with an absorption line–extinction ratio similar to that found in interstellar clouds in our own Galaxy. Extinction could contribute to the large curvature of the spectrum of AO 0235+164. The *UBV* colours of AO 0235+164 would be consistent with those of a typical BL Lacertae object with an additional reddening of ~ 0.5 mag in its $B-V$ colour. Mg II column densities of as much as 10^{+17} cm^{-2} (a possibility suggested by Wolfe and Burbidge[18] for PKS0735+178 for which the doublet is slightly weaker) lead, however, to enormous values of the reddening, if the extinction process is similar to that which applies to the interstellar material in our own Galaxy. For example, ζ Oph, with a Mg II column density of only 10^{15} cm^{-2} (ref. 15) has $E_{B-V} \simeq 0.3$. Also, PKS0735+178 does not appear to be significantly reddened. An intrinsic ultraviolet cutoff in the continuum provides an attractive explanation for the lack of emission lines in BL Lacertae-type sources. AO 0235+164 may be the first such object found at sufficiently high redshift that a cutoff is detectable through *UBV* photometry.

Most of the detected energy from AO 0235+164 was in the infrared and its peak luminosity was $\geqslant 3\times10^{14}L_{\odot}$, if it is at the Euclidean distance implied by the redshift of $z \geqslant 0.852$ ($v/c = 0.55$) and a Hubble constant of 55 km s^{-1} Mpc^{-1}. The total flux density change is 2 orders of magnitude, but its outbursts seem to be relatively infrequent and of sufficiently short duration that the total energy emitted at the lower level of activity is comparable with that released during outbursts. The peak luminosity of AO 0235+164 was $\sim 10^5$ times greater than the luminosity measured for the nuclear source in B2 1101+38[20]. This source has similar properties in other respects: a radio spectrum that is flat or rises with increasing frequency, past large amplitude variability, large and variable intrinsic polarisation, and an absence of emission lines[20,21]. Thus, a single phenomenon seems to operate at power levels ranging from those typical of moderately active galactic nuclei up to those associated with the most luminous QSOs.

Constraints on models

It is generally believed that the radio emission from sources of this type is some form of synchrotron radiation[21]. AO 0235+164 is an outstanding example where the cosmological interpretation of the redshift and the time scale of the variability are very difficult to reconcile with this hypothesis without invoking highly relativistic motion or a special geometry[22].

Attempts to explain the optical and infrared emission as synchrotron radiation encounter even greater difficulties. Except for B2 1101+38 (ref. 23), these sources have not been detected in the X-ray region[24,25] (the pre-outburst Uhuru upper limit for AO 0235+164 is 1.7 × 10^{-10} erg cm^{-2} s^{-1}). To reconcile the lack of Compton-scattered X-ray fluxes with the high surface brightness implied by the rapid fluctuations, very large magnetic fields and very special geometries are required[26,2]. Further X-ray observations during the outbursts of these sources are urgently needed.

Any plausible model for optical synchrotron emission would probably require a sufficiently large magnetic field so that synchrotron self-absorption would occur in the infrared[11]. The behaviour of the spectrum of AO 0235+164 beyond 4 μm and the differences between the radio and optical–infrared light curves could be taken as evidence for self-absorption.

It has not yet been demonstrated, however, that self-consistent models can be constructed along these lines, and our observations of AO 0235+164 strengthen the difficult constraints on solutions to the problem. It is possible that some other, not yet understood emission mechanism is operating, or that these sources are not at the cosmological distances indicated by their redshifts.

We wish to thank Dr H. Spinrad and Dr E. M. Burbidge for sending us their papers in advance of publication. We are grateful to Drs M. Schmidt, P. A. Strittmatter, and R. E. Williams for discussions. We would also like to thank Dr M. J. Belton for making the 0.3-m Schmidt available to us at short notice, Dr D. Wells for technical assistance with the Kitt Peak Interactive Picture Analysis System, and Dr G. F. Benedict for producing the density scans of our spectrograms.

The work of G.H.R. and also that of B.J.W. and D.W. was supported by grants from the US NSF. Kitt Peak National Observatory is operated by the Association of Universities for Research in Astronomy, Inc., under contract with the NSF.

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Received February 16; accepted March 3, 1976.

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Messenger RNA for the coat protein of tobacco mosaic virus

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TMV RNA is not an efficient template for translation of the viral coat protein, in spite of containing nucleotide sequences coding for the protein. Efficient translation requires the prior synthesis within infected cells of a smaller RNA carrying only a portion of the information encoded in the whole genome.

TOBACCO mosaic virus (TMV) consists of an infectious single-stranded RNA molecule 6,400 nucleotides long, encapsidated by 2,100 identical coat protein molecules each with a molecular weight of 17,500 (ref. 1). This packaged RNA (virion RNA) is the sense strand for the translation of coat protein, as was first shown by patterns of mutagenesis[2,3] and more recently by direct nucleotide sequence analysis[4]. The coat protein gene must consist of at least 480 residues of virion RNA. Thus less than 10% of the RNA is accounted for by the coat cistron.

As would be expected, the RNA acts as a good message in cell-free translation systems from bacteria[5], plants[6] and animals[7], but analysis of the products of such incubations has repeatedly shown that coat protein is not the major product, and careful studies with the *E. coli* cell-free system eventually proved that ribosomes never passed over the coat protein cistron in the correct phase[8,9]. This might be due to general incompatibility between eukaryotic and prokaryotic mRNA and ribosomes, but studies of the translation of TMV RNA in various eukaryotic protein synthesising systems have given similarly negative results, thus suggesting that translation of coat protein is not straightforward, and raising the problem of how the coding sequences known to be present on the RNA actually are expressed. In the wheat germ cell-free system, for example, the major products are much larger than the coat protein and also very heterogeneous. Nevertheless Efron and Marcus[6] detected labelled coat protein peptides in products which migrated with authentic coat protein on gel filtration, although they represented only a very minor fraction of the total incorporation. In contrast, when Roberts *et al.*[10] used RNA from the TMV mutant Ni 568, they failed to detect significant levels of product with the mobility of coat protein on acrylamide gels. They did, however, find a coat protein peptide among the products of their incubation, at very low yield, and it was suggested[11] that the coat protein is made as part of a large precursor which is processed by proteolysis to yield mature coat, as in the case of picornaviruses. This idea is inconsistent with the data of Efron and Marcus, who failed to find any coat protein peptides in material larger than coat protein.

We have used two other eukaryotic systems to translate TMV RNA, and have found that it directs the synthesis of discrete polypeptides, which simplifies interpretation (ref. 7 and our unpublished results). In *Xenopus laevis* oocytes[7] and in reticulocyte lysates, the products directed by TMV RNA are two large polypeptides with molecular weights of 140,000 and 165,000; the first corresponds to the largest product of the wheat germ system. We have never detected the synthesis of coat protein. The 140,000 polypeptide does not contain coat protein sequences detectable by tryptic peptide mapping[7] and, given the results reported here, it does not seem likely that the coat is contained within the 165,000 molecular weight polypeptide either. We believe that these proteins are authentic translation products of cistrons in TMV RNA other than the coat cistron, for similar sized proteins are made in TMV-infected leaves and protoplasts[12,13]. In synchronously infected protoplasts, these two proteins are readily detectable about 5 h after infection and there is a further lag of about 4 h before coat protein synthesis begins. Late after infection the synthesis of the two large proteins declines, but coat protein synthesis continues at very high levels. It is difficult to interpret these findings, particularly the latter observation, in terms of a precursor–product relationship between the largest proteins and the coat protein.

It thus seems that there is some temporal and quantitative control of gene expression in TMV, as has been found in essentially all RNA viruses (with the exception perhaps of the picornaviruses), and that most of the extra RNA in TMV which does not code for coat protein codes for the proteins of molecular weight 140,000 and 165,000. If this is correct, and cell-free systems and *Xenopus* oocytes make essentially only 'early' TMV proteins, then how is the coat protein made? We extracted TMV-infected tobacco leaves to see if we could find and characterise the mRNA for

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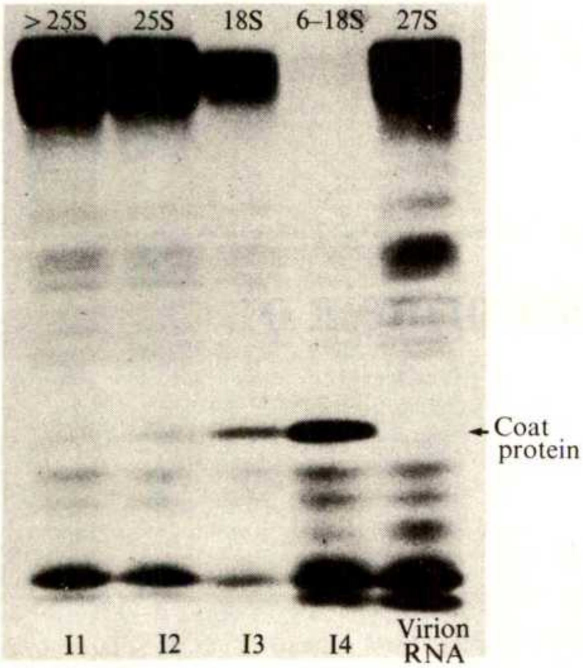

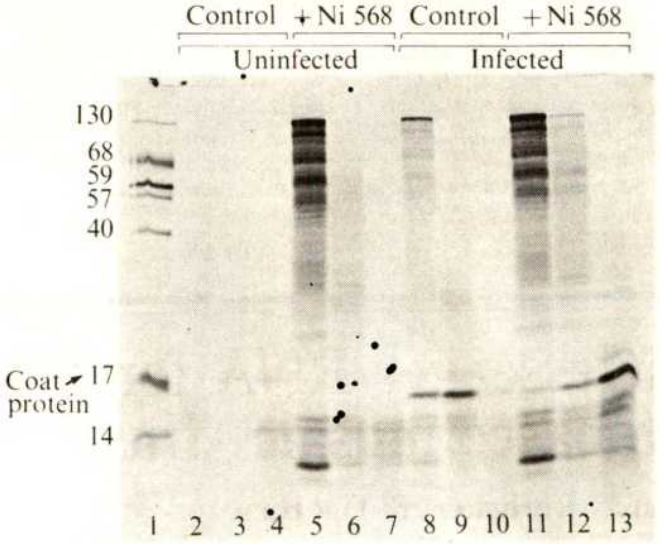

Fig. 1 *a*, Translation of RNA of different size classes from tobacco leaves infected with TMV. Tobacco leaves, infected 7 d previously with TMV vulgare, were washed in water, ground in liquid nitrogen and extracted in an equal mixture of water-saturated phenol and buffer A (10 mM EDTA, 1% SDS, 50 mM Tris–Cl, *p*H 8.8) at 65 °C. The aqueous phase was extracted four more times with phenol, made 0.1 M in NaCl and precipitated with 2 volumes of ethanol. The precipitate was taken up in 1 mM EDTA, 0.5% SDS; warmed for 1 min at 65 °C, and spun at 10,000*g* for 30 min at 4 °C to remove undissolved material. The supernatant was layered over 15–40% (w/v) sucrose gradients in 0.1% SDS, 1 mM EDTA, 20 mM Na acetate, *p*H 5.0, and spun in a Beckman SW 27 rotor for 18 h at 27,000 r.p.m. at 15 °C. The gradients were pumped through a recording spectrophotometer and fractions were pooled as follows: I1 > 25S; I2 ~ 25S; I3 ~ 18S; I4 18S-6S. The RNA fractions were reprecipitated from 0.2 M Na acetate with 3 volumes of ethanol. The precipitates were taken up in water, and stored in liquid nitrogen, except for fraction I4 which was diluted in 1 mM EDTA, 1% SDS, 2 M LiCl and precipitated overnight at 4 °C (ref. 45). The precipitate was recovered by centrifugation at 10,000*g* for 10 min, and taken up in water before ethanol precipitation as above. Each fraction, and RNA extracted from TMV virions, was tested for template activity in a cell-free wheat germ system prepared and incubated as described by Roberts and Paterson[16], except that the ionic conditions in the incubation were: 80 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM spermidine, 20 mM HEPES, *p*H 7.2. The labelled amino acid was L-^{3}H-leucine (20 Ci mmol^{-1}, 10 μM). Reactions were stopped by incubation with pancreatic RNase (100 μg ml^{-1}) for 15 min at 30 °C. Four volumes of SDS gel sample buffer were added[17] and the samples were analysed on 20% SDS-acrylamide gels. After fixing and staining, the gels were impregnated with PPO according to Bonner and Laskey[18] and exposed to Kodak RP Royal X-ray film at −70 °C. The fluorogram shows the products directed by fractions I1–I4, and virion RNA. All RNAs were present at 200 μg ml^{-1} in the incubations. A control without added messenger showed no detectable blackening of the film. *b*, Control for degradation of RNA during extraction. RNA was prepared and fractionated from either uninfected leaves (tracks 2–7) or TMV vulgare-infected leaves (tracks 8–13) as described in (*a*), except that 1 mg of purified Ni 568 virus was added per g wet weight of ground leaves to half of each leaf sample just before phenol extraction (tracks 5–7 and 11–13). The RNA was fractionated by size and the three larger size classes were tested for their template activity as described above, except that products were analysed on a 15% SDS–polyacrylamide gel. The markers in track 1 are β galactosidase, bovine serum albumin, pyruvate kinase, glucose-6-phosphatase, creatine kinase, TMV coat protein and lysozyme, all labelled with ^{14}C-iodoacetic acid.

coat protein, since large amounts of coat protein are made in infected cells, and thus some functional messenger of high efficiency clearly exists. This could in theory be the virion RNA complexed with some control protein, but there were indications from earlier work that the actual messenger might be a smaller RNA[14,15]. This could explain the marked discrepancy between the apparently faithful synthesis of the 140,000 and 165,000 molecular weight polypeptides in *in vitro* systems, and the total lack of coat protein synthesis in the same systems when programmed with virion RNA, implying that the coat cistron in virion RNA is repressed.

Preliminary experiments

RNA was extracted from tobacco leaves which had been systematically infected with TMV 7 d previously. They were frozen and pulverised in liquid nitrogen before extraction in hot phenol–sodium dodecyl sulphate (SDS). After several phenol extractions, RNA was precipitated with ethanol, and after resuspension in aqueous buffer, fractionated on sucrose gradients. Fractions from the gradients were pooled according to their sedimentation rate and recovered by ethanol precipitation. The slowest sedimenting fraction was very heavily contaminated with carbohydrate, and was further purified by precipitation with 2M LiCl at 2 °C overnight. Each fraction, as well as corresponding fractions from uninfected plants, was added to cell-free extracts of wheat germ, and incubated essentially according to Roberts and Paterson[16]. The incubations were analysed by SDS–polyacrylamide gel electrophoresis on slabs[17], followed by fluorography[18] to detect ^{3}H-labelled polypeptides. Figure 1*a* shows the result of one such experiment. A strongly radioactive band with the same electrophoretic mobility as coat protein was made only in incubations programmed with RNA from TMV-infected leaves, and not in incubations programmed with virion RNA. Furthermore, maximal synthesis occurred when the smallest RNA fractions were used, suggesting that the active template was an RNA molecule considerably smaller than intact TMV RNA.

An obvious objection to the straightforward interpretation that a small RNA molecule is the natural mRNA for TMV coat protein is that it might have arisen by degradation of virion RNA during extraction of the leaves. We sought to exclude this possibility by the experiment shown in Fig. 1*b*. The mutant TMV strain Ni 568 contains a single methionine residue in its coat protein[2], unlike the wild-type strain vulgare which contains no methionine. We therefore repeated the extraction of RNA from leaves infected with TMV vulgare, but added a large amount of purified TMV strain Ni 568 virus just before the phenol extraction. After extraction and fractionation of the RNA as before, the fractions were tested in the wheat germ system using either ^{35}S-methionine or ^{3}H-leucine as label in parallel incubations. The products were analysed on slab gels as before; the results of the incubations with ^{3}H-leucine as label are shown in Fig. 1*b*. No coat protein-like products were seen in RNA from uninfected plants, whether or not TMV Ni 568 had been added during extraction. In contrast, such a protein was synthesised when RNA from infected plants was used, but it was labelled only with leucine, and not with methionine. This shows that RNA from the added virions is not converted into a form capable of being translated into the putative coat protein by degradation or modification during extraction of the RNA from leaves. The experiment also shows that uninfected leaves do not contain detectable mRNA for this protein. It should be noted that since typical virion RNA-directed products are observed in the track corresponding to the uninfected plants to which Ni 568 RNA had been added, this RNA was extracted efficiently. Furthermore, the amount of products formed suggested that we had added about the same amount of virion RNA as was naturally present in the infected leaves (compare track 5 with track 8 in Fig. 1*b*). We are therefore confident that the RNA which promotes the synthesis of the putative coat protein is not an artefact of degradation on phenol extraction.

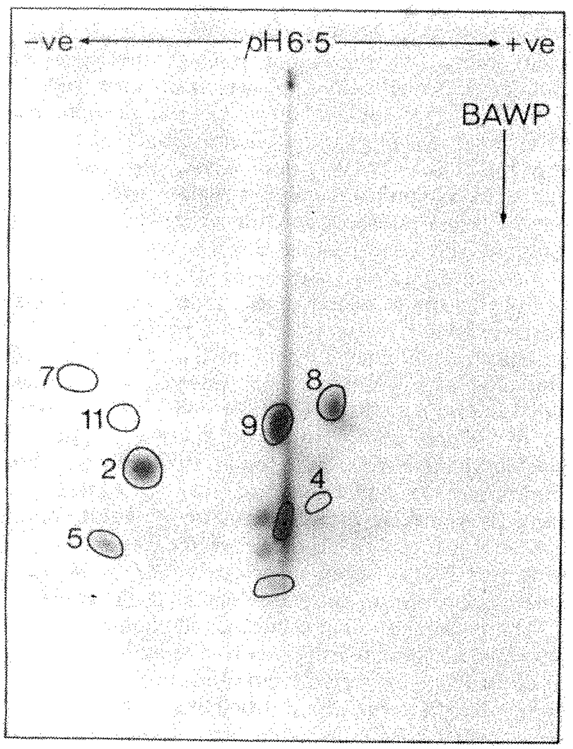

Fig. 2 Fingerprint of putative coat protein synthesised *in vitro*. Fraction I4 RNA from infected leaves was injected into 20 *Xenopus* oocytes, which was incubated in medium containing 20 μCi of L-^{14}C-valine (280 μCi μmol^{-1}) for 24 h. They were homogenised as described previously[7] with 100 μg of authentic TMV vulgare coat protein. The homogenate was centrifuged, and the supernatant fractionated by electrophoresis on an 18% SDS-polyacrylamide gel. The gel was stained with Coomassie blue, dried, and autoradiographed. The stained coat protein band, which was heavily labelled, was cut into small pieces. 2 ml of 10 mM Tris–Cl, *p*H 7.0, 0.1% 2-mercaptoethanol, 2% SDS were added, and the suspension was incubated for 10 min at 95 °C and for 18 h at 37 °C. The bits of paper and gel were filtered off, and the protein was recovered from the filtrate by precipitation with trichloroacetic acid (TCA). It was oxidised and digested with trypsin[7] and the soluble peptides were separated by electrophoresis at *p*H 6.5, 20 V cm^{-2} on a 20-cm^{2} Silica gel G thin layer (250 μm) (Anachem)[46], followed by chromatography in butanol–acetic acid–water–pyridine (30:6:24:20) at right angles to the first dimension. After autoradiography, the carrier TMV coat protein peptides were stained with fluorescamine[47]. The stained peptides (outlined) are numbered from the N-terminus; peptides 4, 7 and 11 do not contain valine.

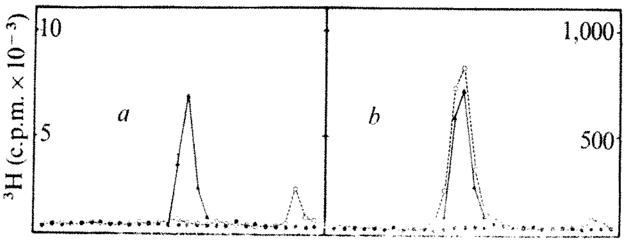

Fig. 3 Assembly of the *in vitro* product. Four cell-free incubations using the wheat germ system programmed with either fraction I4 RNA from TMV-infected leaves or 27S virion RNA, were labelled with ^{35}S-methionine or ^{3}H-leucine. After incubation for 1 h, the ^{3}Hl- and ^{35}S-labelled samples corresponding to each template were mixed together, and 1.8 mg of TMV coat protein was added to both mixtures. The two mixtures were dialysed against 10 mM Tris–Cl, *p*H 8.0, and passed over 0.5-ml columns of DE 52 cellulose equilibrated with the same buffer. After washing with buffer, the coat protein was eluted with 0.2 M KCl, 10 mM Tris–Cl, *p*H 8.0. This material was dialysed against 10 mM Na acetate, *p*H 5.0, to form the helix aggregate[31], which was collected by centrifugation at 100,000*g* for 1.5 h, suspended in Na phosphate buffer, *p*H 7.0, ionic strength 0.1, made 5 mg ml^{-1} in coat protein by a further addition of carrier, and dialysed overnight at 4 °C against the same buffer. The sample was then incubated at 20 °C for 18 h to form disks, at which time TMV RNA (1 mg ml^{-1}) was added, and incubation continued for 10 min at 20 °C. Uncoated RNA was digested with T1 RNase (20 μg ml^{-1}) for 1 h at 20 °C, and the partially reassembled virions were purified by two cycles of preparative CsCl density gradient centrifugation, using added ^{32}P-labelled TMV as marker, in the Beckman SW 50 rotor (CsCl, 1.33 g ml^{-1}, 32 h at 35,000 r.p.m.). The fractions containing marker virus were analysed finally in 8-ml CsCl gradients in the 50 angle head rotor, spun at 40,000 r.p.m. for 24 h. *a*, Result of attempted assembly using the translation product specified by 27S virion RNA; *b*, that using incubations programmed with fraction I4 RNA. Overall recovery of radioactivity from the ^{3}H-labelled incubation programmed with coat mRNA in the peak of CsCl-banded reassembled virus was about 10% while essentially no radioactivity from any other incubation survived to the final analysis. ▲, ^{32}P radioactivity; ○, ^{3}H radioactivity; ●, ^{35}S radioactivity.

Characterisation of the product

The new band programmed by RNA from infected leaves has the same mobility on SDS gels as authentic TMV coat protein, and it lacks methionine. We also found that histidine failed to label the gel band, which would be expected of coat protein, which lacks both methionine and histidine.

When the RNA from TMV-infected leaves was injected into *Xenopus* oocytes, it directed synthesis of a similar band. The yields were higher, and the background lower than in the wheat germ system, and we therefore used oocyte synthesised material to make a tryptic peptide map (Fig. 2). There is excellent correspondence between the *in vitro* synthesised peptides, labelled with ^{14}C-valine, and the fluorescamine-stained carrier peptides, except in the case of coat protein peptides that lack valine. We conclude that this material is chemically indistinguishable from TMV coat protein.

We next asked whether the putative coat protein could be assembled into virus particles *in vitro;* that is whether it resembled coat protein by functional as well as chemical criteria. We therefore partially purified the *in vitro* synthesised coat protein by chromatography on DEAE-cellulose, a procedure which removes basic proteins which may bind nonspecifically to viral RNA during the assembly process. After elution from the column, the protein was concentrated by precipitation at *p*H 5, dissolved in buffer (*p*H 7) and mixed with TMV RNA. After digestion with T1 RNase to remove uncoated RNA tails, the reconstitution mixtures were analysed by CsCl gradient centrifugation with the result shown in Fig. 3. Of four reaction mixtures, using the products labelled with either ^{3}H-leucine or ^{35}S-methionine, programmed by either coat protein mRNA or virion RNA, only the one with coat protein mRNA and ^{3}H-leucine as label gave radioactively labelled nucleoprotein of the same buoyant density as added $^{32}PO_4$-labelled TMV. Furthermore, the labelled protein extracted from this reconstituted nucleoprotein migrated with authentic TMV coat protein on polyacrylamide gels, and no other labelled bands could be detected on the fluorogram. This was in marked contrast to the starting material, which contained many other labelled polypeptides besides coat protein. We conclude from these experiments that a small RNA species present in TMV-infected cells, and not an artefact of extraction, programmes the *in vitro* synthesis of a protein which is chemically and functionally indistinguishable from TMV coat protein.

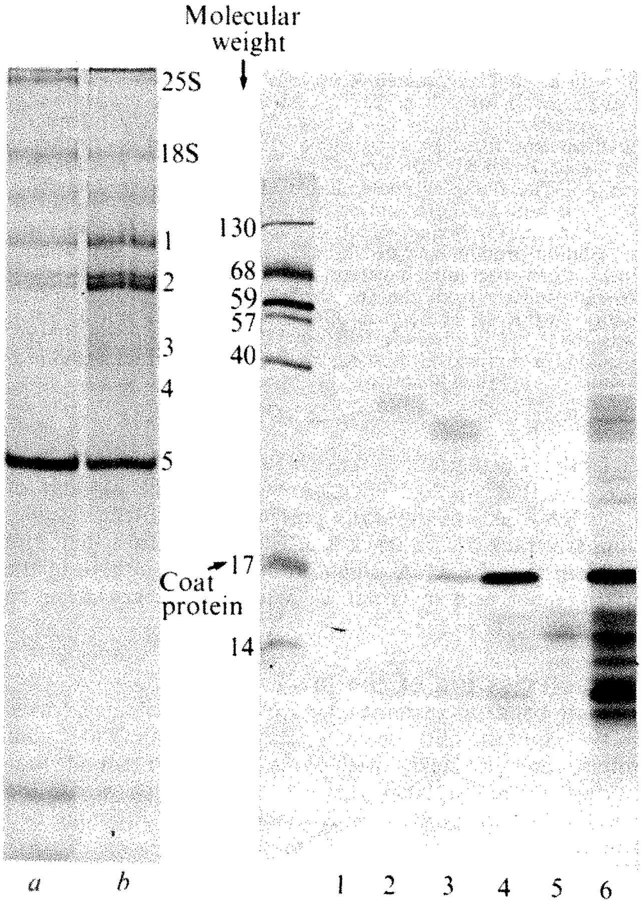

Fig. 4 Fractionation of the coat protein mRNA by gel electrophoresis. On the left are shown two tracks from a 2.8% Agarose/acrylamide gel stained with Stainsall (Eastman Chemicals). *a*, 6–18S RNA from uninfected tobacco leaves; *b*, 6–18S RNA from TMV-infected leaves. The indicated bands from parallel unstained tracks were cut out and the RNA was extracted from them by homogenisation in an equal mixture of phenol and buffer containing 0.1 M NaCl, 10 mM Tris–Cl, *p*H 7.5, 1 mM EDTA, 1% SDS, with 50 μg of carrier yeast tRNA. After extraction for 6 h at 0 °C, the tubes were centrifuged and RNA precipitated from the supernatants with 2.5 volumes of ethanol. The precipitates were washed with ethanol before being taken up in water and tested for template activity in the wheat germ system as described in Fig. 1. The autoradiograph on the right shows the analysis on a 20% acrylamide gel of the products specified by fraction I4 RNA (track 6) and RNA from the indicated gel bands (tracks 1–5). The positions of molecular weight markers are indicated in the left margin of the gel.

Characterisation of the RNA

Up to this point, the fractionation of RNA by sucrose density gradients provided only partially pure coat protein mRNA, but one would expect the coat mRNA activity to reside in a discrete RNA species if it were of genuine biological significance. We therefore took our most active fraction of gradient purified RNA and fractionated it further on an Agarose/acrylamide gel[19]. Figure 4 shows a gel stained with Stainsall[20]; adjacent tracks contain RNA from TMV-infected and uninfected tobacco leaves. We found a reproducible minor stained band in infected leaves which was absent in control leaves. To see if it was coat protein mRNA, a gel with duplicate tracks was run, and one track was stained while the other was cut into bands and the RNA was extracted. We found that RNA from the new gel band was an excellent mRNA for coat protein (Fig. 4). Some of the slower migrating bands also programmed the synthesis of small amounts of coat protein, besides making larger proteins as one would expect. We do not know whether this represents a certain amount of trailing on the gel or of aggregation (since these were not denaturing gels), or whether there may perhaps be larger RNA molecules with coat protein mRNA activity. We estimate that the molecular weight of the major mRNA species is about 2.5×10^5, or 750 nucleotides, of which at least 480 would be required to code for coat protein. Coat protein mRNA failed to bind to oligo(dT)–cellulose (data not shown), showing that, like TMV virion RNA, the coat protein message lacks a significant poly(A) region.

From the classical work mentioned in the introduction, we expected the coat protein mRNA to have the same polarity as virion RNA, and since large end products of RNase T1 digestion which come from the coat cistron have already been characterised[4,21], we sought these characteristic markers in coat protein mRNA. Tobacco plants were infected with TMV and labelled by growing them in medium containing $^{32}PO_4$. Coat protein mRNA was extracted as before, and purified on sucrose gradients and Agarose/acrylamide gels. We found the expected marker oligonucleotides in fingerprints of the T1 RNase digests (Fig. 5*b*), thus proving directly that the gel band contained nucleotide sequences from the expected region of virion RNA, so that it is a fragment of the viral (+) strand, and does not come from an unrelated RNA, such as a satellite virus. There was another unexpected bonus from this analysis, in that the fingerprint bore a striking resemblance to that of an RNA extracted from a nucleoprotein fragment of defined length (containing 1,000 nucleotides) which is generated when TMV particles are degraded in mildly alkaline conditions[22,23]. The RNA from this 'alkali stable fragment' has independently been shown to contain the 3′ terminal T1 oligonucleotide of TMV in molar yield[24], and can be charged with histidine by yeast activating enzymes, as is the case for intact TMV RNA[25]. Analysis of the T1 RNase fingerprint of this fragment showed that it also contained the marker oligonucleotides found in the coat protein mRNA (Fig. 5*a*), showing that the part of the coat cistron from which they originate must be within 1,000 nucleotides of the 3′ end of TMV RNA. Furthermore, the purified coat protein mRNA was found to contain a T1 oligonucleotide which gave rise to the pancreatic RNase products (AAU, C_5,G). This is probably identical with the oligonucleotide AAUCCCCCG which occurs about 20 nucleotides from the 3′ end of intact virion RNA[26]. This suggests that the coat protein mRNA probably corresponds to the 3′ terminal 750 nucleotides of virion RNA.

This assignment of the location of the coat protein cistron is at the opposite end of the RNA to that found by Kado

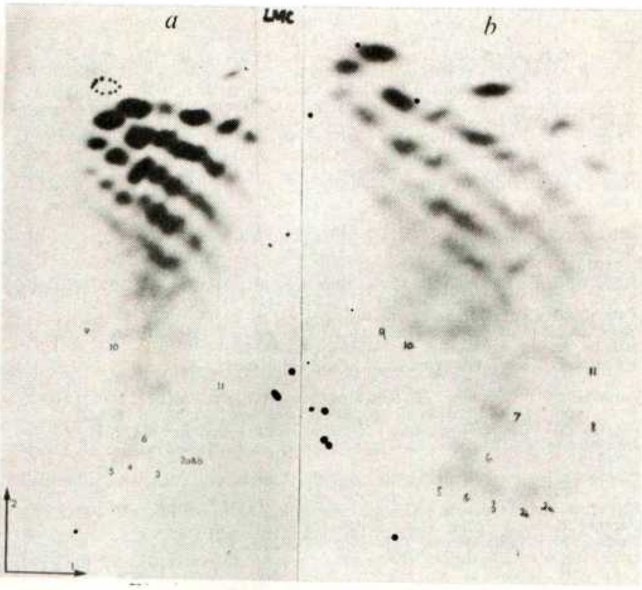

Fig. 5 Comparison of T1 RNase digested coat protein mRNA and 'stable fragment' RNA. ^{32}P-labelled TMV was prepared as described by Zimmern[40] and incubated at 4 °C overnight in 10 mM $NaHCO_3/Na_2CO_3$, *p*H 10.2. Uncoated RNA was digested with micrococcal nuclease (1 μg ml^{-1}) in the same buffer adjusted to *p*H 8.0 with HCl and 1 mM $CaCl_2$. EDTA was added in excess to stop the digestion, and the RNA that had been protected from digestion extracted with phenol–SDS, and purified by sucrose density gradient centrifugation. Fractions containing 'stable fragment RNA' were pooled and precipitated with 2.5 volumes of ethanol. ('Stable fragment' from ^{32}P-labelled TMV was prepared by Michael Wilson.) The precipitate was taken up in 10 mM Tris–Cl, *p*H 7.6, 1 mM EDTA and digested with 3 μl of RNase T1 (1 mg ml^{-1}) in the presence of carrier *E. coli* tRNA (50 μg). The digest was fingerprinted as described by Barrell[44]. Spot 2b is AUAAAUAAUUUAUAGp, which codes for residues 125–129 of coat protein[4]. Spot 5 is AAACCUUCACCACAAGp, which codes for amino acids 53–57[4,21]. Spot 9 is AACCCCACGp, coding for residues 101–103[4]. Spot 4 can also code. Spot 10 here is a mixture, but contains a component giving pancreatic RNase products AAU, Cn, G, chain length 9–10, consistent with AAUCCCCCGp which comes from positions −20 to −28 from the 3′ end of whole TMV RNA (ref. 26). The left hand autoradiograph shows the digest of band 4 extracted from a gel similar to that shown in Fig. 4, except that the RNA was prepared from leaves of plants infected with TMV grown in $^{32}PO_4$. Spots 2a–11 were identified by their pancreatic RNase digestion products as being identical with the equivalent spots in the fingerprint of stable fragment RNA. The more complex appearance of this fingerprint is due to slight pancreatic RNase digestion, which probably occurred during the lengthy extraction process; the mRNA was also slightly contaminated with ribosomal RNA fragments. *a*, Coat protein mRNA; *b*, 'Stable fragment RNA'.

and Knight[27] on the basis of SDS stripping and marker rescue *in vivo*, but agrees with that of Richards *et al.*[28].

Virus replication cycle

On the basis of this evidence, we propose the following account of the replicative cycle of TMV (Fig. 6). After infection of the cell and uncoating of the virion RNA, ribosomes can only translate the cistrons for the 140,000 and 165,000 molecular weight polypeptides, consistent with their early appearance in infected protoplasts. We assume that these functions are required early in infection, and since the subunit molecular weights are close to the apparent native molecular weight of TMV replicase activity in partially purified extracts[29,30], we suggest that both proteins are viral replicases, or their subunits; the function of each being different. For example, one of them may use the (+) strand as a template, the other the (−) strand. Alternatively, one may make coat protein mRNA by partial transcription of the (−) strand, while the other makes full-length transcripts. At least one of them must catalyse the production of complementary copies of the virion RNA, which serve in turn as templates for the synthesis of progeny virion RNA molecules.

The synthesis of coat protein is delayed until its functional mRNA has been produced, because virion RNA does not serve as a template for coat protein synthesis. Two plausible mechanisms for production of the coat protein message are (1) that it is released from virion RNA by specific cleavage, and (2) that it is synthesised by a replicase which initiates transcription at a specific internal site on the complementary strand of TMV RNA. We cannot distinguish between these possibilities at present.

Only when enough coat protein has accumulated to permit the formation of "disk" aggregates[31] can the assembly of virus particles begin[32]. This would delay the encapsidation of virion RNA thereby allowing time for synthesis of early proteins and (−) strands. Once assembly begins the virion RNA will be sequestered and therefore can no longer be a template for either process. But since neither (—) strands nor coat protein mRNA are encapsidated in the case of TMV vulgare, the production of virions can continue for as long as these RNA molecules remain intact, and the replicase(s) active.

This kind of scheme can easily explain how TMV can produce such large quantities of coat protein compared with its other proteins. Being a helical virus, TMV requires many more protein subunits to encapsidate its genome than would a spherical virus with the same genome size[33]. Thus poliovirus, whose RNA is slightly larger than that of TMV, requires only 60 copies[34] of each structural polypeptide compared with about 2,100 in the case of TMV.

Translational control

We do not know why the coat protein cistron is untranslatable in intact TMV RNA whereas it is easily read in the coat protein mRNA; however, at least two precedents offer clues. Initiation of translation of the A cistron of the RNA phages seems to occur only in short growing RNA molecules, since the initiator region of this cistron is involved

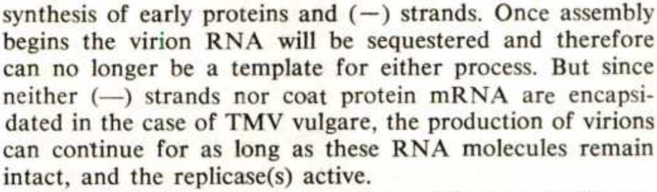

Fig. 6 A tentative map of TMV RNA and its known proteins. The map is drawn to scale. The location of the coat cistron is discussed in the text; the location of the 165,000 and 140,000 molecular weight polypeptides is less certain. Since the sum of their molecular weights exceeds the coding capacity of the virion RNA, they must overlap somehow. We believe they are initiated at different sites since virion RNA can bind two ribosomes simultaneously and independently in the presence of 10^{-4} M sparsomycin, promoting the formation of two methionyl dipeptides: Met–Ala and (probably) Met–Thr (T.R.H. and T.H., unpublished observations). The simplest possibility is that they then terminate at the same site, although this need not necessarily be the case. Their position near the 5′ end of the RNA is suggested by the fact that RNA molecules with small deletions at the 5′ end, constructed by alkali stripping procedures similar to those described in the legend to Fig. 5, fail to synthesise either large polypeptide (T.R.H., M. Wilson, and D.Z., unpublished results). This again is inconsistent with the possibility that the coat protein gene is contained within the large polypeptides (see above). These assignments leave approximately 1,100 residues of RNA unaccounted for, enough to code for up to 40,000 daltons of protein, and this gap has been positioned in the middle of the map. There is evidence from another strain of TMV (the cowpea strain) that a 28,000–30,000 molecular weight protein, whose translation is regulated by a mechanism similar to that described here for TMV coat protein, may be coded in this region[49].

in stable secondary structure in the full-sized RNA molecule[35]. Similar mechanisms may operate in brome mosaic virus (BMV), a multicomponent virus which contains four different RNA molecules. BMV RNA 3 is larger than BMV RNA 4, and just as TMV coat protein mRNA is wholly contained within virion RNA, so BMV RNA 4 is contained within BMV RNA 3 (ref. 36); furthermore, BMV RNA 4 codes efficiently for BMV coat protein, whereas BMV RNA 3 makes another polypeptide but little coat[17]. It seems probable that the coat cistron in BMV RNA 3 is masked by secondary structure, but there is another possibility which may either augment or replace this mechanism. When the sequence of the ribosome binding site of BMV RNA 4 was determined[38], the 5′ end was found to be blocked with m^7G; this unusual end group may be necessary for ribosomes to attach to mRNA[39]. We do not know whether TMV coat protein mRNA has this structure at its 5′ end or not, though intact virion RNA certainly does[40].

Such regulatory mechanisms are not confined to plant and bacterial RNA viruses, however; in both Sindbis and Semliki Forest virus-infected cells small RNAs with the same polarity as the packaged RNA have been found which, unlike their parent molecules, efficiently direct synthesis of the corresponding coat polypeptides *in vitro*[41,42]. Even in the cases of the DNA viruses polyoma and SV40 it seems that larger RNA molecules which code for one protein code for a different protein after cytoplasmic cleavage[43]. Whether such a mechanism regulates the translation of normal cellular messages is unkown. It might account for some cases of 'masked mRNA', for example. We would like to conclude by pointing out that these cases of translational control operate with high efficiency, yet they do not appear to require the action of specific protein synthesis initiation factors.

A preliminary account of these experiments has been published[48].

D. Z. was an MRC Scholar, and T. H. a Beit Fellow. We thank the MRC, Clare College, Cambridge and the Cancer Campaign for research funds, and Jo Butler, Richard Jackson, Bryan Roberts, Ruth Longthorne, Anne Brayley and Christine Harley for assistance. We thank Herman Lehmann for laboratory space.

Received February 3; accepted March 4, 1976.

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letters to nature

Clusters of galaxies as gravitational lenses?

KAROJI and Nottale[1] have compared galaxies situated behind or inside clusters with those whose light does not encounter any important cluster of galaxies. They suggested that either light emitted by distant galaxies is redshifted when passing through clusters of galaxies, or distant sources appear more luminous when seen through intermediate clusters of galaxies, which could act as gravitational lenses. Here we examine the magnitude of the lens effect for clusters of galaxies, and find it unlikely that the observational effect claimed by Karoji and Nottale can be explained in this way.

Consider a source of radiation at a distance $L \simeq cz/H_0$ from the observer, in a universe which is homogeneous on some sufficiently large scale, with mean density ρ_u. The line of sight to the source may, however, be such that the mean density along the beam of light, $\langle\rho\rangle$, differs from ρ_u. The cross sectional area, A, of such a beam of light is governed by the equation[2,3]

$$(A^{\frac{1}{2}})'' + 3\alpha\sigma_0(1+z)^5 A^{\frac{1}{2}} = 0 \qquad (1)$$

where $\alpha \equiv \langle\rho\rangle/\rho_u$ is the density enhancement along the beam, $\sigma_0 \equiv 4\pi G\rho_u/3H_0^2$ is the cosmological density parameter, and ′ denotes differentiation with respect to an affine parameter along the beam of light. The solution of equation (1) for such a 'zero-shear' beam shows that a distant source which would have an apparent magnitude m in a strictly homogeneous universe has an apparent magnitude $m+\Delta m$ when seen along this line of sight, where

$$\Delta m = 1.086\sigma_0(1-\alpha)z^2\{1+O(z)\} \qquad (2)$$

It follows that for $\alpha < 1$, there will be a dimming of distant sources[3]. When looking through a cluster of galaxies, however, $\alpha > 1$ and we expect background objects to be intensified.

To estimate the parameter α when the line of sight passes through a cluster, we consider a cylinder of small diameter along the line of sight, and calculate the mean density, $\langle\rho\rangle$, within that cylinder. Since we are interested in clusters of galaxies, we shall use the approximate density law found by King[4] for the Coma cluster

$$\rho(r) = \rho_c\{1+(r/a)^2\}^{-3/2} \tag{3}$$

where ρ_c is the central density, r is the radial coordinate for the spherically symmetric cluster, and a is the distance at which the density has fallen off to $2^{-3/2}\rho_c$. We assume that the cluster has a radius R, defined to be the distance at which the cluster density fades into the universal background density, that is

$$\rho_c/\rho_u = (1+(R/a)^2)^{3/2} \tag{4}$$

For a line of sight with impact parameter Rh, $0 \leqslant h \leqslant 1$, the density enhancement is then found to be

$$\alpha \simeq 1+2\frac{a\rho_c}{L\rho_u}(1-h^2)^{3/2}/\{1+\left(\frac{h}{u}\right)^2\} \tag{5}$$

where $u = a/R \simeq (\rho_u/\rho_c)^{1/3}$.

The intensification, in magnitudes, is then given by

$$\Delta m(h) \simeq -2.172\,(\sigma_c)\frac{a}{c/H_0}z(1-h^2)^{3/2}/\{1+\left(\frac{h}{u}\right)^2\} \tag{6}$$

where $\sigma_c \equiv 4\pi G\rho_c/3H_0^2$ is the density parameter at the centre of the cluster. It is also of interest to obtain the average intensification, $\langle\Delta m\rangle$, over the area of the cluster

$$\frac{\langle\Delta m\rangle}{\Delta m(0)} = u^2\{(1+u^2)^{3/2}\ln\left(\frac{(1+u^2)^{\frac{1}{2}}+1}{(1+u^2)^{\frac{1}{2}}-1}\right)-2u^2-\frac{8}{3}\} \tag{7}$$

For this approach to be valid, the focal length, $f(h)$, of the gravitational lens must be significantly larger than the source–lens and observer–lens distances, and it must be assumed that the cluster lies roughly halfway between source and observer. The last restriction is not very stringent, for roughly halfway means really that neither the source nor observer lies right next to the cluster when compared to the other.

The angle of deflection for photons passing the lens at a distance Rh from its centre is given by

$$\theta(h) = 4GM_c(h)/c^2Rh \tag{8}$$

where $M_c(h)$ is the mass contained within the cylinder, of radius Rh, whose axis passes through the centre of the lens, parallel to the line of sight[5]. By considering radially adjacent lines of sight with impact parameters Rh and $R(h+\mathrm{d}h)$, the focal length is found to be

$$f(h) = R\left(\frac{\mathrm{d}\theta}{\mathrm{d}h}\right)^{-1} = \frac{c^2R^2}{4G}\left\{2\frac{\mathrm{d}M_c}{\mathrm{d}(h^2)}-\frac{M_c}{h^2}\right\}^{-1} \tag{9}$$

For the King density distribution, we find

$$M_c(h) = 4\pi a^3\rho_c\left\{\ln\left(\frac{(1+(1+u^2)^{1/2})(1+(h/u)^2)^{1/2}}{(1-h^2)^{1/2}+(1+u^2)^{1/2}}\right) - \frac{1-(1-h^2)^{1/2}}{(1+u^2)^{1/2}}\right\} \tag{10}$$

which gives for the focal length of such a lens

$$\frac{f(h)}{f(0)} = \left(\frac{1}{2}\right)\left(\frac{h}{u}\right)^2\left\{1-\frac{(1-h^2)^{1/2}}{1+(h/u)^2}-(1+u^2)^{1/2}\times \times\ln\left(\frac{(1+(1+u^2)^{1/2})(1+(h/u)^2)^{1/2}}{(1-h^2)^{1/2}+(1+u^2)^{1/2}}\right)\right\}^{-1} \tag{11}$$

where

$$f(0) = \frac{(c/H_0)^2}{6\sigma_c a}(1+u^2)^{1/2}$$

is the focal length through the centre of the lens.

If we adopt the parameters given by King for the Coma cluster (noting that he used $H_0 = 75$ km s^{-1} Mpc^{-1}), we then find $\sigma_c = 2.1 \times 10^4$. It follows that for this cluster $f(0) \simeq 750$ Mpc and $\Delta m(0) = -1.94z$, where z is the redshift of the source observed through the cluster. Since the mean radial velocity of the objects found by KN to lie behind clusters is 11,600 km s^{-1}, the average intensification of such an object observed through the centre of the Coma cluster should be $\overline{\Delta m(0)} \simeq -0.076$ mag. Objects not observed through the centre of the cluster will, of course, be less intensified, as indicated by equation (6). If the object being observed lies inside the cluster, there will be a range of α depending upon the position of the object, and hence a greater range of Δm. In fact, a proper treatment of this case would require a detailed knowledge of the parameters of the cluster and the position of the source within it, but we think it unlikely that the maximum intensification would be more than a factor of two different from $\overline{\Delta m(0)}$ above.

Our value of $\overline{\Delta m(0)}$ is considerably less than the mean magnitude differences (-0.26 ± 0.218 and -0.43 ± 0.237) found by Karoji and Nottale, although their error limits are large. It must also be remembered that many of their objects are being seen through clusters which are considerably smaller, and presumably less condensed centrally, than Coma. This suggests that, if the large magnitude differences found by Karoji and Nottale are real, then 'gravitational lensing' is unlikely to be the cause.

Because galaxy magnitudes are notoriously difficult to measure precisely, we suggest, however, that it may be worthwhile to make an accurate, systematic investigation of the apparent magnitudes of a homogeneous, distant sample of galaxies seen through, and outside of, a cluster of galaxies such as the Coma cluster. The general theory of relativity predicts that the lensing effect must be present; our calculation indicates that measurements with an accuracy of ~0.01 mag will be necessary to find it. This is clearly at the limit of accuracy obtainable in measurements of galaxy magnitudes[6]. If the effect were found, however, it would provide not only further verification of general relativity, but also a means of probing the mass of a cluster of galaxies, independent of assumptions about the mass to luminosity ratio of its constituents.

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Received February 19; accepted March 16, 1976.

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Decametric radio emission from four pulsars

We have reported earlier[1] on the reception at low radio frequencies of radio pulses from three pulsars. A similar method[2] was used during 1973–74, and four more pulsars were detected.

The signals were fed into 16 receivers with analog stores giving an equivalent time constant of 100 s. The receiver central frequencies and bandpass values were chosen to allow for frequency dispersion, giving a time resolution of $T/16$, where T is the period of the pulsar. The source was tracked for periods of 1–5 h during the measurement. Stored signals were recorded at intervals of 180–200 s.

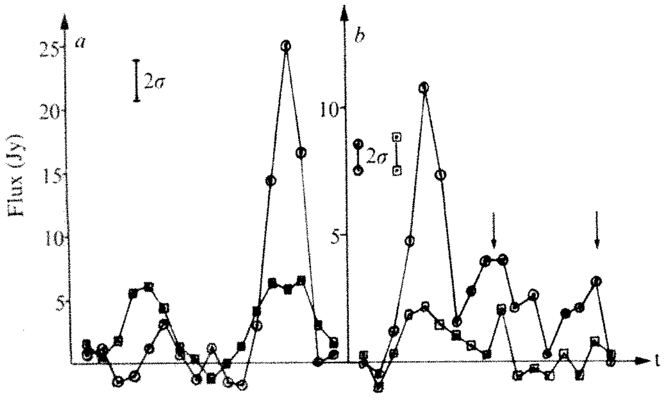

Fig. 1 Integrated pulse profiles for PSR0950+08. *a*, For 1973 March 27, taken over 2,400 pulses. *b*, For 1973 March 1, taken over 23,000 pulses at 25 MHz and 16,000 pulses at 20 MHz. ⊙, 25 MHz data, at 80 kHz bandwidth; □, 20 MHz data at 40 kHz bandwidth.

The signals were averaged over time as well as frequency channels. The signals from the separated frequency bands were superposed with a time delay calculated from known values of the dispersion measure.

At low radio frequencies the integrated pulse profiles are wider than at high frequencies, and they contain new components (interpulses). We distinguished these components from noise by setting a threshold level which depended on the r.m.s. noise level, taking account of the width of the components and the error in determining the mean flux[3]. Some observational results are presented in Table 1, together with the main parameters of the equipment.

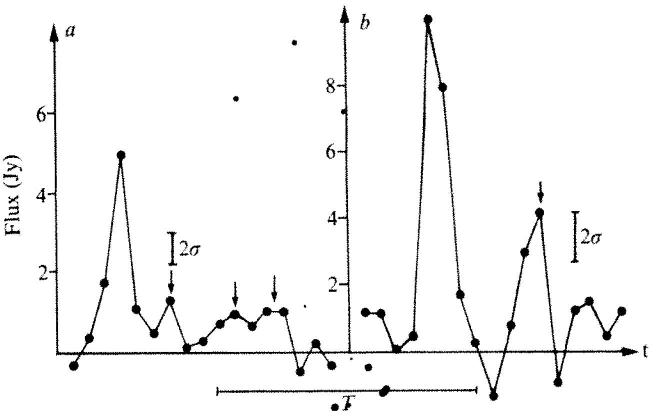

Fig. 2 Integrated 25 MHz pulse profiles for PSR0834+06. *a*, 3,000 pulses on 1973 March 7 and 1973 March 27 at 160 kHz bandwidth. *b*, 1,700 pulses on 1973 April 17, at 80 kHz bandwidth.

PSR0950. As at higher frequencies this pulsar shows a high sporadicity. Shown in Fig. 1 are two typical repeated forms of the integrated pulse profile, recorded at 25 MHz and at 20 MHz. In this and the following figure the 2σ noise level is shown. The main pulse is followed either by one interpulse at $0.4T$ (Fig. 1*a*) or by two at $0.3T$ and $0.7T$ (Fig. 1*b*), which overlap and cover the entire period. This behaviour may be compared with the interpulse observed at high frequencies[4,5], which occurs $0.6T$ after the main pulse.

PSR0834. This pulsar has a lower intensity and it was detected only at 25 MHz. Its integrated profile is very complex (Fig. 2). For 50 per cent of cases it showed an interpulse $0.4T$ after the main pulse; two more interpulses appear rather seldom. The points of minimum emission are stable.

PSR1237. Both this pulsar, and PSR2016, are characterised by a high sporadicity and low intensity. Figure 3 shows two typical

Fig. 3 Integrated 25 MHz pulse profiles for PSR1237+25. *a*, Taken from 1,200 pulses on 1973 January 17: — 110 kHz bandwidth, - - - 110 kHz bandwidth, **—** 220 kHz bandwidth. *b*, 800 pulses on 1973 February 7: — 110 kHz, - - - 110 kHz, **—** 220 kHz bandwidths. *c*, 1973 April 4: — 110 kHz; 1973 April 10: - - - 110 kHz bandwidth; combined data: **—**, 500 pulses. *d*, 1973 March 29: 1,800 pulses; — 110 kHz, - - - 110 kHz, **—** 220 kHz bandwidths.

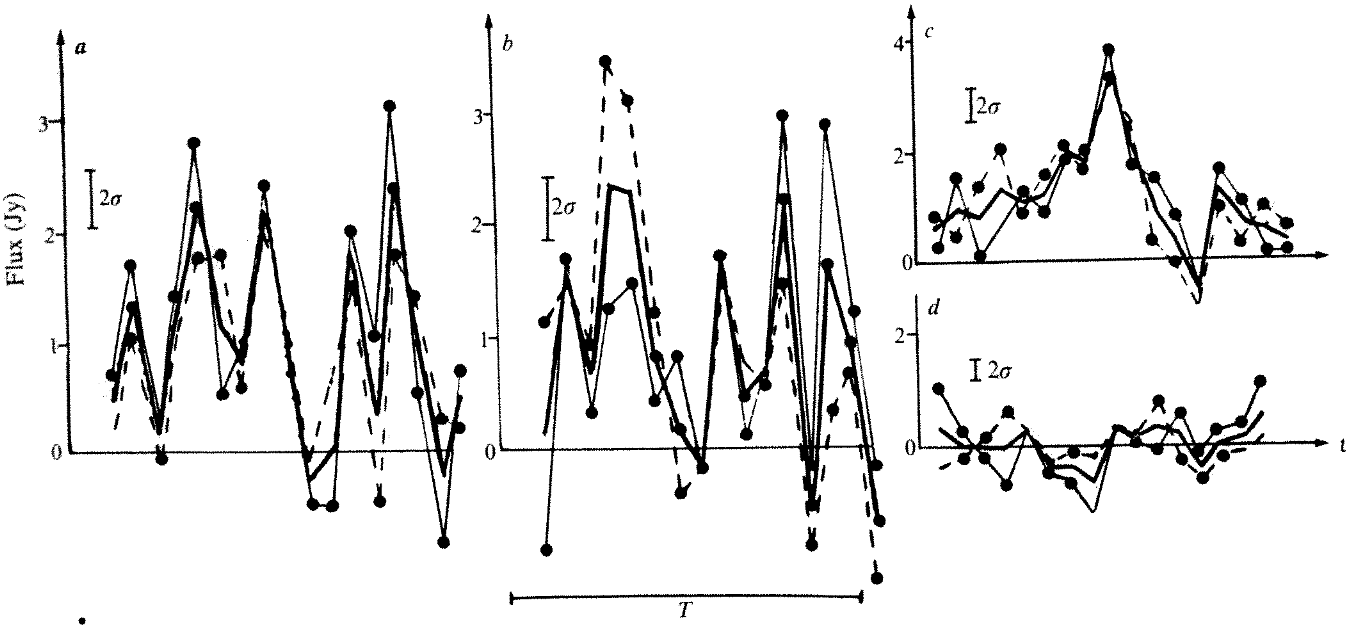

Table 1 Observational results

PSR	Frequency range (MHz)	Band of 1st channel (kHz)	Detuning between the adjacent channel (kHz)	Average flux $\times 10^{26}$ (W m^{-2} Hz^{-1})	No. of interpulses	Width of the main pulse (ms)
0834	25	10	12.00	0.94	0–4	130
0950	25	8–10	9.94	1.7	0–2	35
0950	20	4–5	5.19	0.75	1–2	65
1237	25	14	17.19	1.0	0–4	—
2016	25	4	4.63	0.2	1	35

integrated profiles obtained at different times. Some features are common to Fig. 3*a* and *b*, and to Fig. 3*b* and *c*, but there are obviously very large differences. Figure 3*d* shows a corresponding record when the pulsar signal was not observed.

PSR2016. The integrated profile is shown in Fig. 4. Again the profile is complex, showing a main pulse and an interpulse.

An important conclusion can be drawn from these results and from those of our earlier investigation[1]. The integrated pulse profile, or 'emission window', for all of the seven pulsars so far investigated, covers almost the entire period and contains intense interpulses. This effect seems to be a typical behaviour of the decametric radio band.

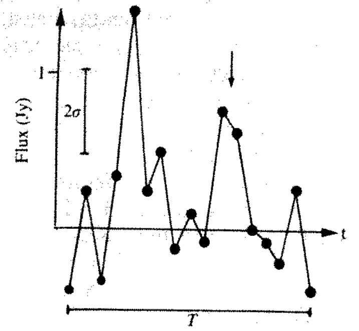

Fig. 4 Integrated 25 MHz pulse profile for PSR2016+28. 10,000 pulses taken on 1972 October 3 at 64 kHz bandwidths.

We thank Professor S. Ya. Brande, Dr N. A. Sapagova and V. C. Sinitsin for their help.

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Received December 1, 1975; accepted February 26, 1976.

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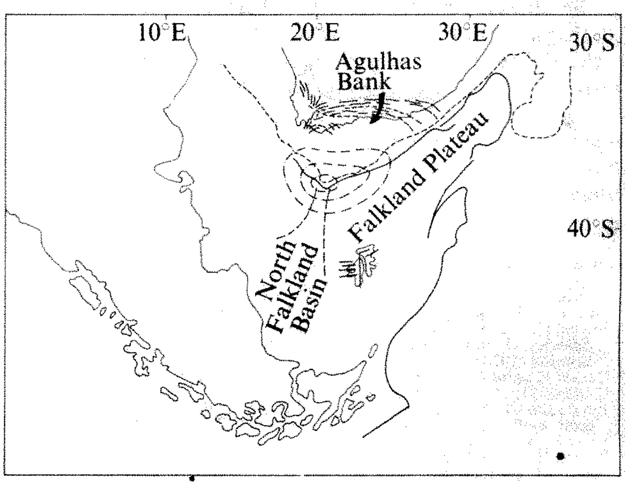
Was there an Agulhas triple junction?

Discussions on the opening of the South Atlantic in terms of major plate tectonic structures such as spreading ridges, transform faults, and so on, have tended to concentrate on the West African segments[1-3]. Evidence has been presented for regarding the original break in the Gulf of Guinea as an RRR junction, the southward-trending arm becoming the Mid-Atlantic Ridge, the north-east-trending arm (Benue Trough) undergoing a relatively minor phase of opening followed by closing, and the westward arm becoming modified by a series of oblique shears so that movement there became a combination of spreading and transform motion (ref. 1, Fig. 1). An analogous set of movements was proposed for the Red Sea, Dead Sea, Gulf of Suez junction. Later, a large number of other triple junctions was recognised[4], including a Georgetown RRR junction to the west which helped to define the course of the Africa/South America split and left the Takutu rift in Guyana as a failed arm filled with Cretaceous deposits.

So far, little attention has been paid to the southern end of the Atlantic spreading ridge in the reconstructions of the breakup of Gondwanaland, although the presence of a major transform (the Agulhas/Falkland Fracture Zone) is now well established. Little is known in detail about the Falkland Plateau, but the presence of the Falkland Islands, and the granitic basement found by deep drilling[5] strongly suggest that the whole plateau consists of continental crust. Assuming this to be the case, and taking the 2,000-m isobath as defining the plateau edge, it is possible, using a slightly modified Bullard rotation, to fit the Falkland Plateau into the embayment of the distal Natal Valley, between the Agulhas Fracture and the southern Mozambique Ridge, with very little gap or overlap (Fig. 1). The South Atlantic spreading ridge and the Agulhas Fracture meet at approximately a right angle, and as Burke and Dewey[4] have suggested that such angular bends commonly mark the site of triple junctions, it is worth enquiring whether a third arm exists. There is, in fact, evidence of a trough almost bisecting the angle made by the two other fractures; this is the North Falkland Basin, the present-day trend of which is north-east, and which lies north-west of the Falkland Islands. This contains[6] up to 2 km of sediments, mainly of suggested late Cretaceous age.

Thus it is possible to postulate a triple junction consisting of the Atlantic spreading ridge, the Agulhas Fracture, and the North Falkland Basin—that is, a spreading ridge, a transform fault, and a failed arm—and it is proposed that this be called the Agulhas Triple Junction

Fig. 1 Proposed Agulhas Triple Junction, showing schematic contours of uplift (–·–) and relationship to the Cape Fold Belt.



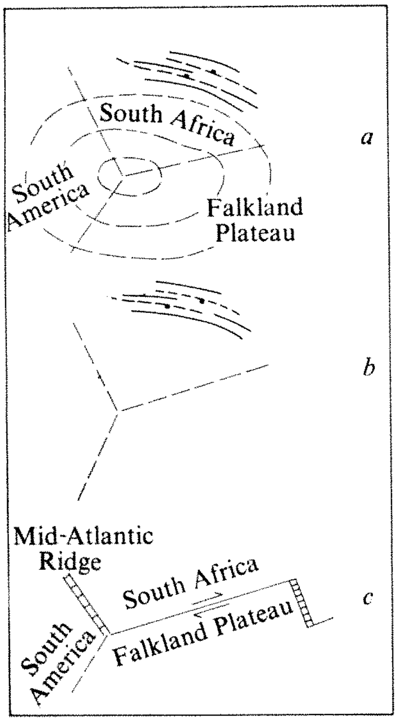


Fig. 2 Schematic representation of the proposed evolution of the Agulhas Triple Junction. *a*, Uplift and doming, radial and concentric fractures, formation of the Cape Fold Belt (early Triassic); *b*, subsidence with reversal of throw on Cape faults, no spreading, (?) initiation of Mesozoic basins (Triassic to Jurassic); *c*, initiation of separation, further faulting in Cape Fold Belt (early Cretaceous).

(Fig. 1). The similarity to the examples of the Gulf of Guinea, Georgetown and the Gulf of Suez is clear.

The idea of a possible Agulhas triple junction arose from studies in the Cape Fold Belt[7], which resulted in the proposal of a gravity sliding model for the development of that orogen. This required that uplift should take place south of the present Fold Belt to form the slope on which sliding was initiated, and it was found that uplift centred on the proposed junction was well situated to do that. Since the initiation of triple junctions seems to depend on the strain developed over an uplift, probably resulting from a rising mantle plume[4], it seemed natural to link the two concepts (Fig. 2). Upwelling must have been followed by subsidence (since no spreading occurred at this stage), which led to major southward downthrow on the large longitudinal faults associated with the Cape Fold Belt, and possibly also to the initiation of the Mesozoic basins of the South African continental shelf and coastal areas, which contain sediments as old as Triassic[8]. Separation only occurred later (125–130 Myr)[9] but could have been controlled by the directions of the earlier-formed fractures.

I thank Professor R. V. Dingle for drawing my attention to the existence of the North Falkland Basin, and to C. J. Hartnady for the Bullard reconstruction.

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Received October 27, 1975; accepted March 17, 1976.

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Shortening of continental crust in orogenic belts and plate tectonics

THE geotectonic and palaeogeographic problems created by severe crustal shortening in orogenic belts are numerous and difficult to solve. In spite of plate tectonics, or because of it, one such problem of major significance persists: where is much of the continental crust that once underlay many of the nappes in tremendously shortened and imbricated belts such as the Alps[1–4]? In the scheme of plate tectonics, it is implausible to pull, melt or shove continental crust back into the mantle[5]; yet some authors express faith in such a concept[6] or, lacking faith, despair of a solution[7]. I propose that the solution to this problems lies quite simply in the fact that the original eugeosynclinal basement[8] of the Alpine belt consisted of distended continental crust, as clearly understood by Trümpy[9].

It is possible to calculate the crustal shortening in the Eastern Alps by assuming constant crustal volume through time. The present crustal volume may be measured from a crustal model of the Eastern Alps based on detailed gravity and seismic studies[10]. In agreement with Dewey *et al.*[11], I assume that the volume of continental crust measured in the Alpine geophysical profiles is a reasonable approximation of the original volume of continental crust that existed before development of the Tethys and the Pennine Trough as well as before Alpine deformation. Crustal additions and subtractions from and by the mantle, the oceanic crust, and erosion and sedimentation (and also by volume changes) are thus considered relatively insignificant or mutally cancelling for the purposes of my calculations. The assumption of no crustal addition may not be far fetched, at least for the eastern Alps, considering the paucity of Mesozoic–Cainozoic magmatism and ophiolites in the area. The assumption of no crustal subtraction by erosion is clearly wrong for the Alps, but the evaluation of erosional losses would only amplify the conclusions of this study, and thus erosion is ignored. The assumption of no crustal subtraction by 'oceanisation' or disappearing continental blocks is reasonable, and is the principal point shown here.

Figure 1 shows the total measured crustal volume of a 1-km-thick slice of the 222-km-long profile to be 9.640 km^3. with an average crustal thickness of 43.4 km. (For this calculation, made with a polar planimeter, I have subtracted out the chemical contribution of the volume of the Mesozoic carbonate rocks in the Northern Limestone Alps.) Assuming an initial normal crustal thickness of 30 km, it follows that an initial length of section of 321 km would be required to maintain constant volume. This would require a shortening of nearly 100 km in the present state. This number does not compare with geological estimates of 180–600 km of shortening[1,3,4] of the palaeogeographic realms underlain by continental crust in the Eastern Alps.

The Red Sea region can be used as a valid geotectonic model of the Triassic–Jurassic Alpine belt, representing an initial crustal configuration in which palaeogeographic realms are sited on distended continental crust (see refs 12 and 13). From the profile (Fig. 2), a simple analysis as described in Fig. 3 results in the values shown in Table 1.

Table 1 Parameters from Red Sea and Alpine regions

Length of Red Sea profile	449 km
Shorten profile to subtract all oceanic crust	−82 km
Shorten to restore continental crust to original thickness (30 km thick)	−146 km
Shorten to thicken continental crust to 43.4 km average of present Eastern Alps	−69 km
Final width of the profile	152 km

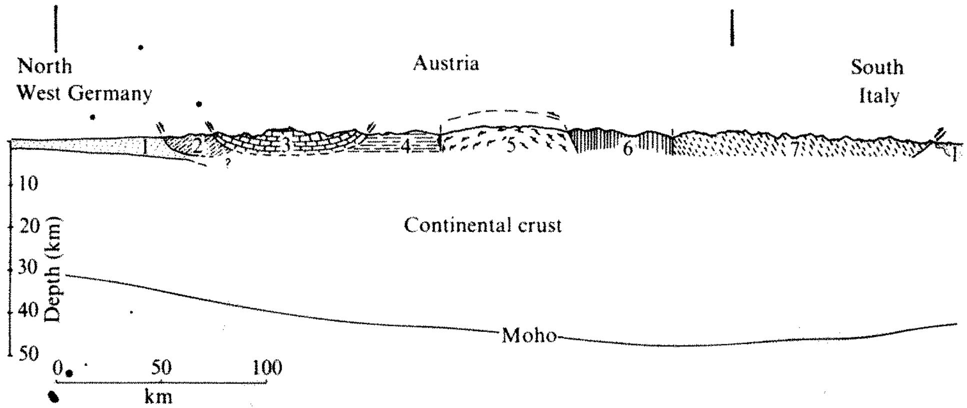


Fig. 1 Crustal profile through the eastern Alps from near Chiemsee, West Germany (north), in the molasse zone, to Maniago, Italy, in the Po Basin (adapted from Angenheister *et al.*[10]). 1, Molasse; 2, flysch and Helvetic zone; 3, Northern Limestone Alps; 4, greywacke zone; 5, Tauern fenster; 6, Altkristallin; 7, Southern Alps.

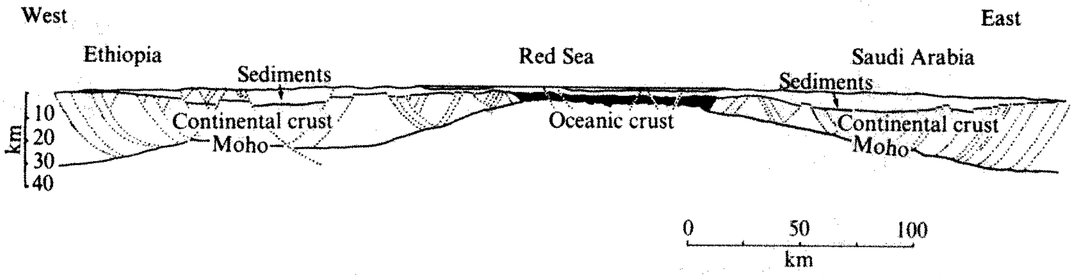


Fig. 2 Crustal profile through the southern Red Sea from Ethiopia (west) to Saudi Arabia, based upon gravity, seismic, magnetic and bore hole data (adapted from Lowell and Genik[12]). The extreme thinning of continental crust shown in this profile is not agreed upon by all workers, but is considered to model the Alpine region in the Triassic–Jurassic. This profile is not entirely perpendicular to regional strike. In calculations, the sediments are not included in the volume of continental crust in as much as they are largely chemical precipitates.

Assuming the effective removal of the oceanic crust without trace, the shortening of the continental crust is 215 km.

In order to attain the final dimensions of the Alpine profile for direct comparison, I have added 70 km of length to reach 222 km (Fig. 1) Since this 70 km must have a final thickness of 43.4 km, it is actually necessary to add 100 km of crust 30 km thick to the initial profile (Fig. 2). That is, the Alpine crustal profile balances in volume with a Red Sea profile that includes an extra 100-km-long stretch of normal continental crust beyond that shown in Fig. 2. Therefore, the final dimensions of the crustal shortening model developed are as shown in Table 2.

Table 2 Dimensions in the crustal shortening model

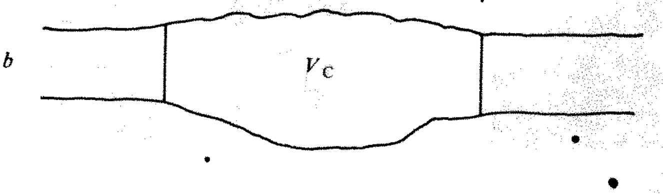
State	Dimensions of profile
Initial stage of continental distension (Red Sea model)	549 km long (82 km oceanic length) 9,640 km^3 per km continental crust Continental crust varies from 0 to 30 km thickness
Final stage of orogenic shortening (Eastern Alps)	222 km long 9,640 km^3 per km continental crust Continental crust 43.4 km average thickness 245 km of crustal shortening on continental basement

This model of crustal shortening, though based on gross and simplifying assumptions, seems to provide an explanatory step in the right direction. For, along with a geologically reasonable estimate of crustal shortening over continental basement (245 km), it also eliminates the need for disappearance of large volumes of continental crust, a concept distasteful to fundamental physical principles. The problem of excessive crustal shortening in orogenic belts may, therefore, be eliminated on consideration of the simple fact that initial thinning of continental crust of orogenic belts has not until now been taken into account in models of crustal shortening.

Of course, I am aware that many factors contribute to changes of crustal volume and that the deformation of colliding continental blocks, in particular, must be complex in space and time[14–16]. This paper serves only to emphasise the critical importance of initial crustal configuration when considering problems of orogenic crustal shortening. The application of this result to the study of orogenic tectonic evolution will be of interest.

I am grateful to Professor A. Tollmann for his assistance and for providing a Gastdozent position at the University

Fig. 3 Method of calculation. The crustal volume in profile of two rifted continental margins (*a*) is set equal to the crustal volume of the orogenic profile (*b*). Knowing the length L_2, L_1 can be calculated to satisfy the balance $V_A + V_B = V_C$. The amount of shortening on continental basement is $L_1 - L_2$. The horizontal dimension of any oceanic crust in profile (*a*) is eliminated because it and its overlying sediments (except chemical sediments that must be subtracted) cannot enter into the crustal balance as discussed in the text.



of Vienna where this study was done. Among numerous colleagues, I must especially thank P. Faupl and D. Roeder for discussions.

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Biostratigraphy of some marine manganese nodules

Using scanning electron microscopy (SEM) we have examined the microtexture of Mn nodules. Previous researches using SEM observation on marine Mn nodules have revealed many characteristic features which may relate to their formation[1–6].

We report here the discovery of *in situ* calcareous nannoplankton fossils in a Mn nodule collected during the RV Hakuho-Maru Cruise KH-74-4, and present a preliminary calculation of the growth rate, using a biostratigraphic method. We also discuss the potential value of Mn nodule biostratigraphy[7] in terms of deep sea palaeoenvironments.

The Mn nodules were dredged from a brown clay by a Norwalk type dredge at the summit of Makarov Guyot (29°26.3′N, 153°27.3′E; water depth, 1,378 m)[8]. One of them (16002) is spheroid in shape, with concentric symmetric layers around a basaltic rock nucleus (Fig. 1*a*). The crust, about 60 mm thick, comprised three layers: an outermost, 2 mm thick with a bumpy surface texture (Fig. 1*b*); a middle, about 8 mm thick, comprising many thin lamellae; and the innermost, about 50 mm thick, massive and dense in texture.

The outermost layer and the innermost layer adjacent to the nucleus were peeled off by hand after drying at room temperature, and were coated with carbon and gold thin films for SEM observation.

The ooze adhering to the surface of the outermost layer comprised calcareous nannoplankton fossils: *Coccolithus pelagicus* (Wallich) Schiller; *Cyclococcolithina leptopora* (Murray and Blackman) Kamptner; *Emiliania huxleyi* (Lohmann) Hay and Mohler; *Gephyrocapsa oceanica* Kamptner; and *Umbilicosphaera mirabilis* Lohmann. The assemblage represents NN21 zone (0.2 Myr–present)[9].

The surface of the outermost layer itself is also covered with calcareous nannoplankton fossils. The microtexture and the presence of *E. huxleyi* (Fig. 1*c*) indicate the recent growth of the nodule.

The inner surface of the outermost layer also contains calcareous nannoplankton fossils preserved as impressions (Fig. 1*d*) or replaced by ferromanganese oxides (Fig. 1*f*). Although most are too poorly preserved, several species have been identified: *C. pelagicus; Cyclococcolithina macintyrei* Bukry and Bramlette; *Cyclococcolithina leptopora; Pseudoemiliania lacunosa* (Kamptner) Gartner; and *U. mirabilis*. The assemblage indicated NN19 zone (2–0.5 Myr). The presence of a coccosphere of *Cyclococcolithina leptopora* (Fig. 1*d*) suggests that the fragile coccosphere itself may have been covered with ferromanganese oxide soon after the deposition on to the nodule surface, which subsequently dissolved away after consolidation of the oxide. Likewise, the other microfossils are considered to have been rapidly incorporated into the layer, probably simultaneously with the ferromanganese oxide. Besides the late Neogene calcareous nannoplankton fossils, several individuals of *Chiasmolithus grandis* Hay, Mohler and Wade (Fig. 1*e*), an Eocene index species, were found inside a cavity in the outermost layer. Since, unlike the other *in situ* microfossils, they were observed only in the cavity,

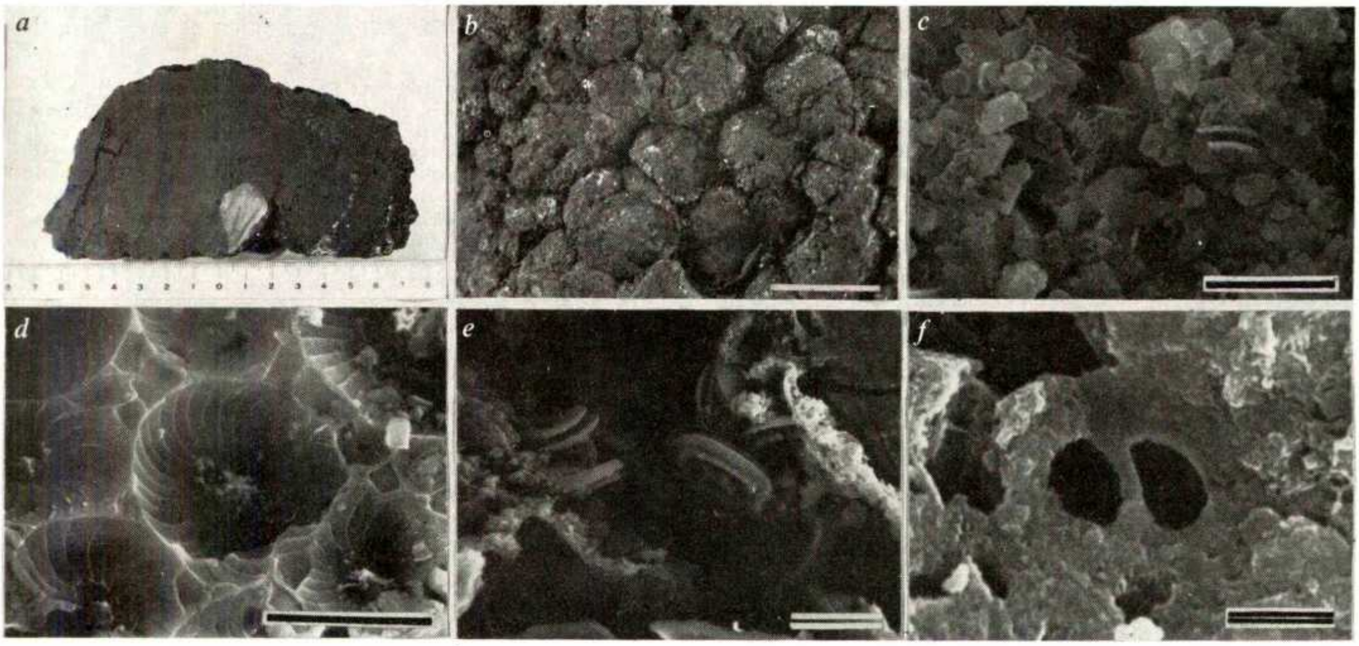

Fig. 1 Photographs (*a*, *b*) and SEM photomicrographs (*c*–*f*) of a marine Mn nodule. *a*, Tangential section showing three major layers: thin outer layer; middle layer with many thin lamellae; and massive innermost layer (scale, 1 cm). *b*, Detail of the surface texture of the outermost layer. Bumps were peeled off for SEM observation (scale, 1 cm). *c*, Enlarged surface texture of the outermost layer after ultrasonification. *E. huxleyi* embedded in the layer indicates the recent growth of the nodule (scale, 5 μm). *d*, Impression of a coccosphere of *Cyclococcolithina leptopora* suggesting that most of the microfossils were incorporated into the layer rapidly (scale, 5 μm). *e*, *Chiasmoliuths grandis* (scale, 20 μm). *f*, *G. oceanica*, replaced by Mn oxides, in the innermost layer adjacent to the nucleus (scale, 1 μm).

apparently unaltered, they may have resulted from the reworking of the sediments.

In the innermost layer adjacent to the nucleus, *G. oceanica* (Fig. 1*f*), which first appeared at the beginning of the Pleistocene, were observed. No specimens of *Discoaster*, a species which became extinct at the end of the Pliocene, were found. Greenslate has suggested an important role for siliceous microfossils in nodule formation[4]; however, we have not recorded significant amounts of siliceous fossils (see refs 5 and 6).

Since the calcareous nannoplankton fossils preserved in the layers record the age of each layer, we can calculate the mean growth rate of each layer; other methods[10–14] only give that of the outer layer.

The assemblages of the outermost layer indicate that it started to grow 2–0.5 Myr ago, and that it continued at least until 0.2 Myr ago. The mean growth rate of the outermost layer is thus 1.0–6.7 mm Myr^{-1}. The values calculated are compatible with those obtained by conventional methods: 1–100 mm Myr^{-1} (ref. 15).

On the other hand, the presence of *G. oceanica* and the absence of the members of *Discoaster* in the innermost layer adjacent to the nucleus suggest that the nodule is younger than 2 Myr old. Therefore, the minimum mean growth rate of the nodule, except for the outermost layer, is 39 mm Myr^{-1}. That seems reasonable, though it is about 5–40 times higher than values obtained from the outermost layer. The massive texture of the innermost layer may reflect a rapid growth. Further study of the intermediate layers is needed to verify the hypothesis.

The discrepancy between the two growth rates suggests an irregular rate of accretion not considered in previous work[12]. This hypothesis is supported by the nature of the surface texture and by the presence of coccospheres. As the accretion rate of marine Mn nodules is considered to relate intimately to low sedimentation rates and to the water current on the sea floor[15], the drastic change of rate may correspond to a change in the deep-sea environment in the region during the late Pleistocene.

If the assemblage of microfossils in the layers of Mn nodules can be correlated with those in the sedimentary sequence of the same region, more information on both the origin of the nodules and on the regional palaeoenvironment of the deep sea should be provided.

This work was financially supported in part by the Ocean Research Institute, University of Tokyo. We thank Professor N. Nasu for offering the opportunity to join the Hakuho-Maru Cruise KH-74-4, Dr E. D. Goldberg, Scripps Institute of Oceanography, for help with the manuscript, and Professor T. L. Ku, University of Southern California, for critical comments.

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Climatic implications of stable carbon isotopes in tree rings

STABLE carbon isotope variations in tree rings are normally attributed to either a direct temperature dependent fractionation process in the tree[1], or alternatively to variations in atmospheric composition[2,3]. To clarify this apparent conflict we have begun measurements of ratios in rings of Southern Hemisphere trees. In spite of some obvious differences between the two trees studied so far, both show a positive correlation with regional temperature trends over the past 75 yr. This result is discussed in terms of the alternatives above, and with the view of extracting palaeoclimatic information. Verification of such influences could provide > 2,000 yr of data on the climate of the region.

Five-year blocks were taken from two King Billy pines (*Arthrotaxis selaginoides* D. Don) which grew within 200 m of each other in natural forest in mountains of northern Tasmania (Fig. 1). A total of 71 separate burns were made on the 25 5-yr blocks of one tree and the CO_2 from each burn analysed at least twice. The δ^{13} variations for each block were generally < 0.1‰. The second tree had 31 5-yr blocks and 50 burns were performed. A full discussion of the experimental technique, tree environment and dendrochronology, and raw data will be published elsewhere.

Variations of 0.2–0.3‰ in δ^{13} are observed in consecutive blocks but these are not always duplicated in the two trees. On a longer time scale, however, similar major trends are evident in both trees, a decrease in δ^{13} from 1895 to ~ 1945 and a rapid increase back to the 1895 level by 1970. This is

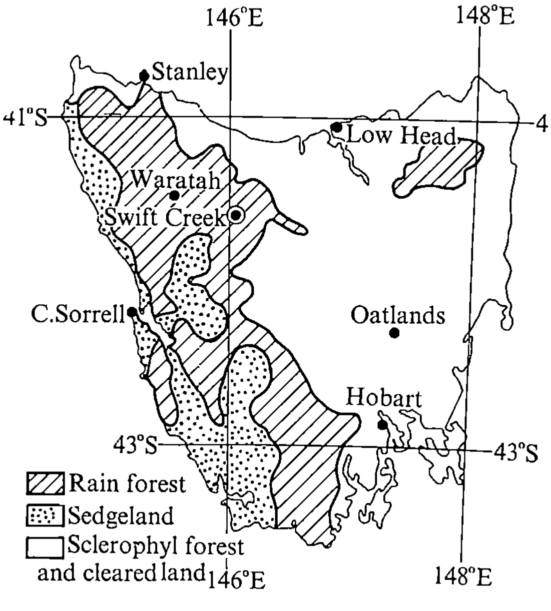


Fig. 1 Map of Tasmania showing the location of the six climatological stations and the Swift Creek site from which the study trees were obtained. Representative samples of 25–75 mg from each block were burnt in an oxygen atmosphere similar to that described in ref. 4 The resulting CO_2 was cryogenically trapped and its isotopic composition analysed using a Micromass 602B mass spectrometer. Results are reported in conventional δ^{13} units, where

$$\delta^{13} = \left[\frac{(^{13}C/^{12}C)_{\text{wood}}}{(^{13}C/^{12}C)_{\text{standard}}} - 1\right] \times 1{,}000(‰)$$

A cylinder of CO_2 was used as a standard and by adding an arbitrary −8.0‰ our data were made comparable to the PDB standard The influence of solvent extraction, cellulose separation and variations in oxygen isotopes were found to be of similar magnitude to those reported elsewhere[5–8], relatively constant throughout heart and sapwood, and incapable of significantly influencing the long term trends observed. Thus the bulk of the measurements were on whole wood samples.

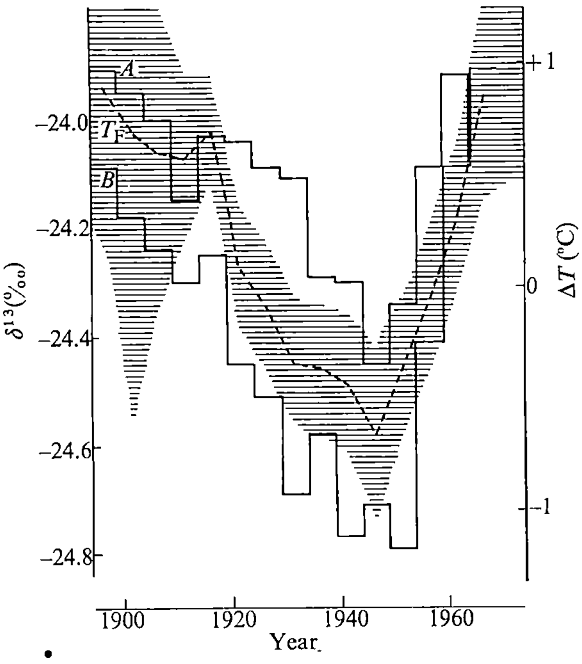
illustrated in Fig. 2 which gives δ^{13} histograms for each tree. The large block-to-block variations have been partly suppressed with a 3-block (15-yr) running mean.

Corresponding 5-yr blocks (means) of ring width, rainfall and temperature data have been analysed and correlated with the δ^{13} curves. The only significant correlations (5% significance level as indicated by the Student's t test) are with maximum temperatures. The correlation coefficients improve with the 3-block smoothing, and also consistently and significantly as the temperature data are restricted to summer months, particularly February. Convective mixing in the lower atmosphere during the daytime ensures that maximum temperatures are far less susceptible to local influences than are minimum (and thus mean) temperatures. Thus maximum temperatures will be more influenced by, and therefore more highly correlated with, the mean temperature of a larger volume of the atmosphere, particularly in summer, when convective activity is greatest. It may also be important that January and February maximum temperatures more closely represent times of tree growth. In Fig. 2 the pecked curve represents the 3-block running mean of February maximum temperatures for six stations surrounding the tree location (Fig. 1). The consistent long term behaviour of all stations (and, by implication, the tree site) is indicated by the shaded area which encompasses the standard deviations obtained by averaging the normalised deviations from the mean for each station. Similar temperature trends are suggested by data of Coughlin[9] for 9 provincial towns in SE Australia, by sea surface measurements east of Tasmania (D. J. Rochford, personal communication), and also by recent data for New Zealand[10].

A regression analysis of δ^{13} against temperatures for the curves of Fig. 2 gives temperature coefficients of 0.48 ± 0.12 and $0.24 \pm 0.11\%\ ^{\circ}\mathrm{C}^{-1}$ for trees B and A respectively. The correlation between unsoothed temperature and mean tree data is significant to the 1% level.

A temperature dependent fractionation process during photosynthesis with a theoretically derived coefficient of $0.36‰\ ^{\circ}\mathrm{C}^{-1}$ has been proposed by Libby[12], and supported by a comparison between the δ^{13} trends in a German oak and Western European temperature variations. The comparison is far less convincing than those in Fig. 2 and gives a temperature coefficient which is now known to be overestimated[7]. Laboratory investigations of a temperature fractionation effect in non-arboreal species[2,11] generally show an effect very much smaller and of opposite sign to those reported here.

Fig. 2 Smoothed deviation (ΔT) from the long term average of February maximum temperature for six Tasmanian stations and the δ^{13} of trees A and B.



Freyer and Wiesberg[2,13] have summarised their own extensive measurements[7], plus those of Farmer and Baxter[3], relating to δ^{13} values in Northern Hemisphere tree rings. From trees selected as representing carbon exchange with the free atmosphere, a common δ^{13} pattern has emerged. It falls ~0.5‰ between 1905 and 1940, rising ~0.3‰ by 1960 and falling 0.3‰ thereafter. Both sets of authors propose that the trees are registering variations in the isotopic composition of the atmosphere. Dilution of atmospheric ^{13}C by the release of biospheric CO_2 accompanying increased land cultivation is identified with a sharp fall early in the century, and fossil fuel combustion accounts for only a small overall decrease. Climatic variations possibly acting through ocean and biomass exchange are mentioned as possible contributing factors. Correlation coefficients between the tree ring data of Fig. 2 and those described above are ~0.5, and scarcely significant at 5%. Over the years 1905 to 1940–50, however, the similarity is more pronounced.

Much of the δ^{13} variation and the similarity could be accounted for by CO_2 exchange with the temperate oceanic mixed layer as the result of temperature changes. Freyer and Wiesberg[2] calculate a change in δ^{13} in the atmosphere of +0.3‰ for each 1 °C temperature increase. The exchange would be dominated by long term temperature variations in the Southern Hemisphere whose oceans contain 80% of the total temperate mixed layer. The regional temperature variations shown in Fig. 2, however, may not be representative of the hemisphere because of a lack of spatial coherence of temperature variation on a sub-hemisphere scale[14]. For the same reason, we cannot dismiss the possibility that similarities in δ^{13} variations in both hemispheres may have resulted from the fortuitous occurrence of similar temperature trends in the regions from which trees were collected. The Freyer and Wiesberg and the Farmer and Baxter trees may have responded directly to regional temperature trends which were different to those accepted for the Northern Hemisphere as a whole[15].

Whatever the cause, the tree analyses reported here show a significant correlation with regional temperature on a time scale longer than 5–10 yr. We are hesitant to accept a single explanation in terms of a direct temperature effect on photosynthesis, because on occasions changes in δ^{13} differences between the two trees would require up to 2 °C changes in the 5-yr averages of the temperature differences between sites 200 m apart. It is possible that the local canopy significantly influences the isotope ratio either by temperature effects of shading or by an isotope enrichment process as discussed by Keeling[16], with indirect temperature links. It is anticipated that information from a wider selection of trees, on both longer and shorter time scales, will resolve these questions.

The assistance of Dr D. Adamson of Macquarie University, who provided the dated timber, and Drs W. Budd, V. Morgan and Mr G. Wilson of the Australian Antarctic Division, who provided facilities and expertise for the mass spectrometry, is acknowledged.

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Received February 25; accepted March 18, 1976.

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Spontaneous measurement by young children

WHETHER or not the young child can make transitive inferences ($A>B,\ B>C\ \therefore\ A>C$) is still a controversial issue. Bryant and Trabasso[1] have claimed that he can, provided that he remembers the necessary information, but this claim has been disputed[2,3]. Youniss and Furth's main argument is that it is one thing to show that a child can make inferences, but quite another to demonstrate that he puts these inferences to any effect. They cite the measurement experiment of Piaget *et al.*[4] as evidence that the child does not make inferences spontaneously to solve problems. Here children had been shown a tower of bricks on a table and asked to build one as big on the floor. A stick the same height as the first tower was available. the question was whether the child would use this as a measure to equate the towers, thus making an inference ($A=B,\ B=C\ \therefore\ A=C$). Children below 8-yr old did not measure with the stick, and it was concluded that they had no idea of measurement. We here report experiments which lead to the opposite conclusion.

An alternative interpretation to Piaget's is that children do understand that it is possible to use intervening measures, but did not in this case realise that a direct comparison of the two towers by eye might be unreliable and thus that some measurement was needed. We decided to test this notion in three experiments in which, as the two quantities to be compared were invisible, it was very obvious that a direct comparison was not viable. We predicted that here children would measure.

Our experimental test involved 4 trials; in each the child was given two identical looking black wooden blocks (8 inch high, 3 inch wide) side by side on a table. Each had a hole in the top, the complete depth of which could not be seen: the hole was either 6 or 4 inch. In 2 of the 4 trials the holes were the same (6 inch with 6 inch, 4 inch with 4 inch): in the other two one hole was 6 inch, the other 4 inch

Between the two blocks lay a 10-inch long stick: its central 2-inch portion was yellow while the surrounding 4 inch at either end was red, so that the yellow portion was uncovered when placed in a 4-inch hole and covered in a 6-inch hole.

In each trial the child was asked to find out whether the two holes were the same and, if not, which was the deeper. We wanted to know if the child would spontaneously use the stick as a measure to make this comparison. If he did not on any trial he was given a prompt to use the stick as a measure.

In experiment 1, 20 5-yr olds and 20 6-yr olds were also told on each trial to use the stick though they were not told how. In experiment 2 the same number of 5 and 6-yr olds were told nothing about the stick. In experiment 3 the same number of 5 and 6-yr olds were given the same task as in experiment 2, except that the entrance to the two holes was widened and painted white, to see if this would distract the child from measuring. (The depth of the holes was still invisible.)

Also in each experiment each child was given a pre- and post-test version of Piaget *et al.*'s measurement task, which was exactly the same except that it was miniaturised, with a toy table and towers and sticks which were either 6 or 4 inch.

All the children failed both traditional tower measuring tests in all three experiments. Many of them were, however, successful in our hole measuring task. As Table 1 shows, the majority of children used the stick as a measure without any prompting, and most of their judgments were correct. A substantial number of children were never prompted and were correct on every trial. There were no significant differences between age groups or experiments. We conclude that young children do have an idea of measuring.

In experiment 4 we tested our idea that a crucial factor is whether or not a child realises that a direct comparison will be unreliable with 40 5-yr olds and 40 6-yr olds. Half had to compare holes in Perspex boxes. To make direct comparisons of the visible holes unlikely we changed the depth of the holes in this experiment to 6 and 5.5 inch and we placed the blocks on different tables in both conditions. Otherwise the procedure was the same as before.

The prediction was that Perspex boxes, by encouraging direct comparisons, would lead to less measurement. Although the results (Table 1) provide some partial support for this prediction, the difference was not significant. Many children still measured even in this experiment.

Whatever is the importance of the child's appreciation of the precariousness of direct comparisons, we have established contrary to all preceding evidence that young children can spontaneously use intervening measures. This result has some educational significance and since the measuring must have involved inferences it is strong support for the claim that young children make, and benefit from making, transitive inferences.

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Table 1 Performance in the four experiments

	Expt 1		Expt 2		Expt 3		Expt 4 Wooden box		Expt 4 Perspex box	
	5 yr	6 yr	5 yr	6 yr	5 yr	6 yr	5 yr	6 yr	5 yr	6 yr
Mean age (yr. months)	5.6	6.6	5.6	6.5	5.8	6.4	5.5	6.6	5.7	6.8
No of children (out of 20) measuring on every trial with no prompt	17	19	15	14	16	16	11	16	7	14
No. of children never prompted and correct on all 4 trials	10	15	10	9	11	10	8	7	2	7
Mean no. (of 4) of correct judgments by those children never prompted	3 41	3.79	3.53	3.43	3.38	3.50	2.84	2.91	3.07	3.44

Territory size, breeding time and mating preference in the Arctic skua

THE Arctic skua is a polymorphic seabird with three phenotypes in plumage—pale, intermediate and dark. The dates during the breeding season when new pairs breed are related to the phenotypes of the males[1,2]: on average, dark males breed before intermediate males who breed before pale males. This effect is most pronounced when the male has no previous breeding experience[3]. These observations can be explained by assuming that females have mating preferences for the darker males[2,4]. Each breeding pair of Arctic skuas defends a territory: some defend their territories very strongly, attacking intruders viciously, others seldom attack In the red grouse, androgen levels have been shown to determine both aggression and mating success of males[5]. We show here that the size of a male's territory may either correlate with breeding time and mating preference through a common cause, or determine them both directly.

Since 1973 we have studied the colony of Arctic skuas on Fair Isle This study has included the accurate surveying of the positions of the nests of all pairs of birds in the colony We have mapped the nests on paper, drawn the lines connecting each nest with its adjacent nests and then the lines perpendicular to, and bisecting, each of the connecting lines. This divides the area of the colony into polygons, each enclosing a nest Parts of the colony are occupied by great skuas as well as Arctic skuas The great skuas' nests were also mapped, since great and Arctic skuas show interspecific territoriality. We consider the area of each polygon to be a measure of the size of the territory. When territories are sharply bounded in a dense colony, they naturally tend to become polygonal[6]. Only pairs with territories surrounded by others were included in the following statistical analysis. The full details of this method of mapping territories and the statistical analysis will be described in a subsequent paper.

In the data from those males who are mating with a new female and who have previous breeding experience, the hatching date is negatively correlated with territory size. The larger the territory, the earlier the breeding date. The correlation is very significant statistically (see Fig. 1: $r=-0\ 462$; $N=35$; $P<0.01$) There is no significant correlation if the males are without previous breeding experience ($r=-0\ 182$; $N=33$), and no correlation at all if they are breeding with the same female as in previous years ($r=0\ 015$; $N=100$). The overall correlation for males breeding with a new female, however, is very significant ($r=-0.334$; $N=68$), and the difference in the correlation for these males is not significant whether they have previous breeding experience or not ($\chi^2=1\ 544$).

Available evidence and casual observation at the colony suggest that the pairing of males with some previous experience of breeding may be different to the pairing of those with no experience. All the experienced males who changed their mate, bred on the same territory as they had done in previous years. Their new mate was often a female who had also bred nearby in the past. Immature males may find their mates differently, however. The immature birds return to the colony in the years before breeding, forming loose groups, or 'clubs', in particular parts of the colony. The inexperienced males probably select their mates from among the females of the club having taken over a territory during the year before breeding. Since the experienced males start the season with a territory, breeding time is a good measure of the time taken to find a mate. The inexperienced males, however, may have already paired with a female and defended a territory before breeding in the following year: if so, they would not form a group comparable to experienced males seeking a new mate, and breeding time would not be so closely related to territory size; the correlation is in fact reduced from −0.462 to −0.182, but, as we have already stated, this reduction is not significant given our present sample sizes.

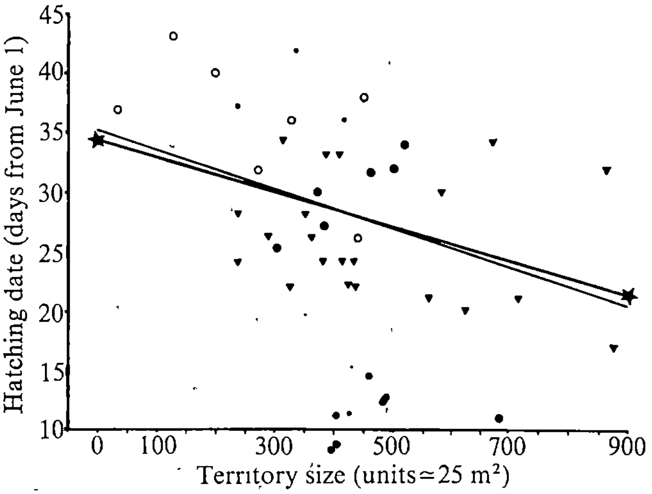

Fig. 1 Relationship between territory size and breeding time among males with previous breeding experience who are mating with a new female. A computer simulation, assuming that females have probabilities of mating proportional to the sizes of territories of males who are still unmated, gives the regression $y = 34.48 - 0\ 01455x$ (★—★) which is very close to the observed regression $y = 35.52 - 0.017x$ (———); ● Dark; ▼ intermediate; ○ pale

With the data available, we can only speculate on the cause of the correlation in the males between breeding time and territory size The following hypotheses would explain the observations:

(1) Females may simply prefer males with larger territories. We cannot see how this hypothesis can be tested.

Table 1 Fitting of parameters of negative assortative mating to the data of Murton *et al.* on matings between phenotypes of feral pigeons

Parameters fitted	Values of the fitted parameters at maximum likelihood	Values of χ^2	Degrees of freedom
None	—	50.869	6
α	0.340	40.945	5
β	0.508	26.856	5
γ	0.397	35.243	5
α β	0.327 0.520	8.271	4
α γ	0.355 0.416	10.782	4
β γ	0 508 0.376	6.565	4
α β γ	0 000 0.508 0 376	6.565	3

In the model, the females have preferences α, β and γ to mate with the phenotypes 'wild type', 'blue checker' and 'dark-blue checker'. The preferences are expressed only when the male's phenotype is different from the female's. Also included in the data were rare birds with other phenotypes. These were grouped together as 'others' and it was assumed there were no female preferences in their favour.

(2) Territory size may determine both breeding time and mating preference. Females will be more likely to land by random choice on the larger territories. The male with the larger territory will therefore pair earlier in the season. Such an effect would certainly account for the correlations we have observed. Given the data of Fig. 1, we have computed for each female the probabilities of mating proportional to the sizes of territories of males who are still unmated. This gives a correlation coefficient of -0.393 which is not significantly different from the observed correlation. Unfortunately this simple and satisfying hypothesis cannot be the complete explanation of our observations· as we have already said, when males change their mates,· the females usually come from nearby territories and not at random. Nonetheless, some of the females may be arriving to land on the territories at random, and if the darker males have larger territories this would partly account for their earlier average mating time and the mating preference in their favour. Sexual selection would thus favour increased territory size since earlier pairs have greater reproductive success.

(3) Territory size and breeding time may both be determined by a common cause. Territory size may be a measure of the intensity of breeding behaviour It would then be related to the mating preferences of females for the different phenotypes of males. O'Donald, Wedd and Davis[2] and O'Donald[4] estimated the mating preferences for intermediate and dark males according to a number of specific models of mating preference. The best estimates suggested that ~29% of females preferred intermediate males and 18% preferred dark males: these were the values that gave the closest fit by maximum likelihood. On Fair Isle, intermediates mate assortatively. A model of assortative mating gives an estimate of the mating preference for intermediates which agrees with that obtained from the distributions of breeding times[7,8]. The mating preference for dark males is only slight and not statistically significant, but this is perhaps to be expected in view of the small number of dark males in the sample. If the mating preferences are determined by sex drives of the males, we should expect that the intermediate and dark males would also have the larger territories; they do, but not significantly so, for our present sample contains only few pale males (see Fig 1). Our data are fully in agreement with the expectations of this hypothesis but to test it more rigorously further observations are required, both on territory size and its changes among pale, intermediate and dark males, and on the behaviour in the clubs of immature birds.

No correlation has been observed between territory size and breeding time in pairs that have bred together in previous years. There is also very little variance in the breeding dates of particular pairs in successive years[9]. The large territories tend, however, to get smaller and the small territories tend to get larger ($r=-0.698$; $N=21$; $P<0.001$) This could easily be explained if territories in successive years were random samples of available territories. Territories are not, however, allotted each year at random· the same pair always keep the same territory, whose size is not expected to vary since the average territory size in a particular part of the colony does not vary much from one year to the next. Perhaps larger territories take more defending since they are more likely to be disturbed by intruders. If the effort of defending a territory has evolved to an optimum by natural selection, a pair with a larger territory may, therefore, allow it to contract during the breeding season and so start with a smaller territory next year.

An interesting comparison between melanism in the Arctic skua and melanism in feral pigeons gives further support to our third hypothesis Murton, Westwood and Thearle[10] have shown that the melanic blue checker and dark-blue checker pigeons have larger testes during the breeding season and remain longer in breeding condition than wild-type pigeons. Pigeons show negative assortative mating of their phenotypes, however, unlike the positive assortative mating of the Arctic skua. We have analysed these data using a model of negative assortative mating. In the model, the females have preferences α, β and γ to mate with wild-type, blue checker and dark-blue checker males. These preferences are not expressed when the males have the same phenotypes as the females. (In the model of positive assortative mating for the Arctic skua[7], the mating preferences are expressed only when the males have the same phenotype as the females.) Table 1 shows the result of fitting our model to the data of Murton *et al* The females clearly prefer the melanic males. In pigeons, this is likely to have a physiological explanation since the larger testes of the melanics imply higher levels of gonadotropins and more intense sexual activity. It would be interesting to see if there are similar physiological differences between melanic and non-melanic Arctic skuas. Such differences would explain both mating preferences and the correlation between territory size and breeding time, but would not explain the assortative mating Positive assortative mating can evolve as a result of linkage disequilibrium between the genes for the preferences and those for the preferred phenotypes[11]. Negative assortative mating could evolve only as a result of some specific selective advantage.

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Received December 29, 1975. accepted March 8, 1976.

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Retrograde amnesia following electroconvulsive therapy

AMNESIC agents, such as electroconvulsive stimulation, can cause loss of memory for events that occurred before treatment[1]. Usually as the interval between learning and convulsive treatment is increased, the resulting retrograde amnesia is diminished[1–3]. This temporal gradient of retrograde amnesia can sometimes cover several years[4]. Clinical descriptions of the amnesic syndrome suggest that information about the temporal sequence of events can be more severely impaired than other aspects of memory[5,6]. Thus, an amnesic patient may describe a past event accurately but be unable to report when the event occurred. We have administered a new remote memory test based on former one-season television programmes to psychiatric patients receiving bilateral electroconvulsive therapy (ECT), and report here that memory for temporal order is markedly affected by ECT. ECT caused retrograde amnesia for order information about programmes broadcast from 1 to ~7 yr before treatment, but not for programmes broadcast from 8–17 yr before treatment.

The subjects were 17 female and 3 male psychiatric inpatients 27–64 yr old (mean=45 yr), for whom a course of bilateral ECT had been prescribed for relief of depressive

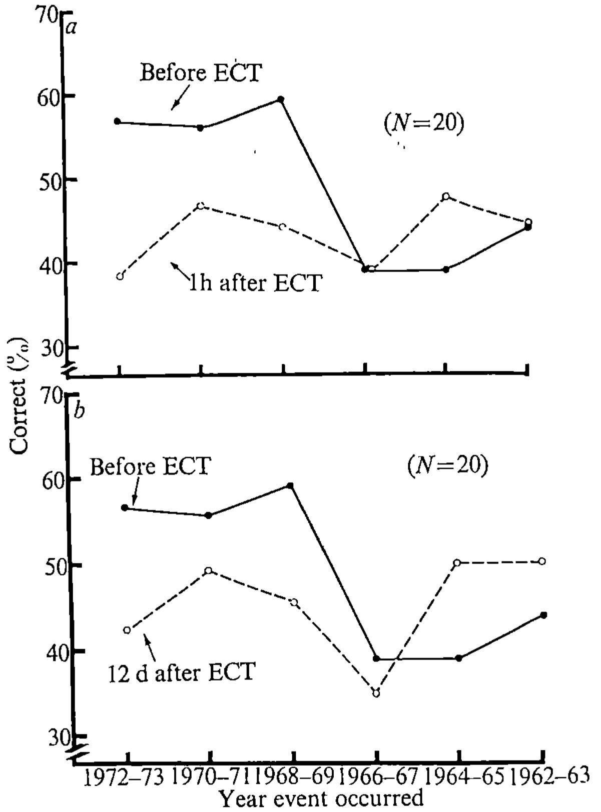

Fig. 1 Results of the test requiring temporal judgments about remote events administered to depressed psychiatric patients receiving a course of bilateral ECT. Testing was conducted before ECT, 1 h after the fifth treatment (*a*), and 1–3 weeks (mean = 12 d) after the completion of treatment (*b*). The test asked patients to select from sets of 3 former television programmes the one that was broadcast most recently. Comparisons were always between a programme broadcast for one season between 1962 and 1973 and two programmes broadcast for one season 5 years earlier. The time indicated is that when the correct choice was on the air. Values >42% are significantly above the chance level of 33% ($t>2.1$). ECT selectively impaired performance for the time period 1968–1973.

illness. Four of these 20 patients had received one course of ECT from 3 months to 14 yr previously. ECT was given with a Medcraft (B-24) machine (140–170 V, 0.5–1.0 s), with temporal–parietal electrode placement. This treatment was given three times per week on alternate days under barbiturate anaesthesia (methohexital sodium, 60–120 mg), and atropine (0.8 mg), with full muscular relaxation (succinylcholine, 20–60 mg).

The temporal judgment test was based on previously described recognition tests[7,8] involving the names of 85 one-season television programmes, 5 from each year 1957–1973. Popular exposure of the programmes from each year was relatively similar, and names were apparently learned close to the time the programmes were on the air. A three-alternative, multiple-choice test of 36 questions was constructed that repeated the question: "Which television programme was on the air most recently?" The correct answer was always the name of a programme that was broadcast for one season from 1962 to 1973. The two incorrect choices were the names of programmes broadcast 5 yr before the correct choice, 1957–68. The order of the 36 questions was random with respect to the periods they covered. All patients took this test 12–18 h before their first treatment, 1 h after their fifth treatment, and 6-25 d (mean = 12 d) after completion of their course of ECT (5–18 treatments, mean = 9.0). Testing was conducted from October 1974, to August 1975.

Figure 1 shows performance on the temporal judgment test before and after ECT. Before ECT, patients performed similarly to a control group[8] of 30 hospital volunteers ($F=0.1$, $P>0.3$). Apparently performance on this test was not noticeably affected by depressive illness. One hour after the fifth ECT (*a*), the performance was markedly impaired. The effect of ECT on 1968–73 scores accounted for 90% of the variance in before–after scores, and the before–after difference was significant for 1968–69 ($F=4.8$, $P<0.05$) and 1972–73 ($F=7.0$, $P<0.05$).

The results cannot easily be explained by gross confusion or by an inability to obtain high test scores after ECT. First, verbal IQ is normal by 1 h after 5 bilateral treatments[4,9]. Second, ECT did not simply reduce the frequency of high scores across all time periods. For the three periods not affected by ECT (1962–1967), patients obtained 15 scores of 67% or better before ECT and 14 scores of 67% or better after ECT. Thus, the impairment after ECT cannot be attributed to a global impairment in the ability to perform well, but is consistent with the interpretation that ECT selectively affected memories acquired in recent years. This conclusion is also supported by the fact that after ECT the score for 1972–73 was lower than the score for any other time period and was not significantly above the chance level of 33% ($t=0.9$, $P>0.3$).

Figure 1*b* shows that the impairment in temporal judgment for remote events persisted 1–3 weeks after completion of treatment. At this time the effect of ECT on 1968–1973 scores accounted for 71% of the variance in before–after scores, and the before–after difference was significant for 1968–69 and 1972–73 ($F>9.0$, $P<0.01$). A comparison of performance 1 h after the 5th ECT with performance 1–3 weeks after the completion of ECT indicated that no recovery had occurred during this period. The scores were nearly identical on these two occasions ($F=0.7$, $P>0.3$) and did not differ by >6% for any period.

These results indicate that ECT markedly disrupted the ability to make temporal judgments about remote events. Compared to amnesia for the names of former programmes[4], retrograde amnesia for temporal information about these programmes was more extensive and persisted for a longer time after treatment. Thus, amnesia for names of former programmes covered the past 1–3 yr, whereas amnesia for temporal information covered ~1–7 yr. Memory for the names of past programmes had recovered by 1–2 weeks after a course of ECT (mean = 7.6 treatments)[4], whereas judgment of temporal relationships between these programmes was still impaired 1–3 weeks after ECT (mean = 9.0 treatments). A long term follow-up study is now in progress to determine how long after ECT the disturbance of judgment remains.

Our results, together with previous experimental findings[4,10] and clinical case reports[11,12], support Ribot's hypothesis[13] that the resistance of memory to disruption increases with time. These results differ from findings that ECT[9] or Korsakoff psychosis[14] caused retrograde amnesia for past public events that affected all past time periods almost equally. Ageing also appears to affect memory for past public events equally across all time periods, rather than to cause a greater loss of memory for more recent time periods[15,16]. These studies do not, however, provide strong evidence for or against Ribot's hypothesis. First, the performance of Korsakoff patients[14] was so close to chance for questions about past time periods that a tendency for performance to be affected differently could easily have been masked. Indeed, in a subsequent study using easier questions, Korsakoff patients did exhibit a selective retrograde amnesia for more recent time periods[10]. Second, in the study of remote memory it is difficult to compare results for different times unless efforts are made to eliminate possible sources of sampling bias[15]. Third, performance of normal subjects was usually about the same for all times (1930–1969)[9,15], possibly because the absence of questions about the most recent years precluded the appearance of a forgetting curve. In contrast, normal performance on questions about former television programmes, including questions about the immediately preceding years, was much better for recent periods than for more remote periods[7,8]. One interesting possibility is that the development of resistance to amnesia parallels the course of normal forgetting. By this view, differential resistance to amnesia should not be demonstrable in the asymptotic limb of a forgetting curve.

Taken together, the evidence supports the conclusion that the resistance of memory can develop for several years after learning, and that temporal order information is particularly vulnerable to disruption. The neural substrate of memory may change over the years so that material in memory becomes both harder to recall and more resistant to disruption.

We thank the staffs of Mesa Vista, Vista Hill, and Encinitas Hospitals for their full cooperation. This work was supported by a grant from the National Institute of Mental Health and by a Clinical Investigatorship from the Veterans Administration.

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Development of visual stimulus-seeking in dark-reared kittens

EARLY visual experience is important for the development of vision in kittens. The response specificity of visual neurones for different environmental features can be affected by selective visual exposure to those features, and leads to long term changes in visual capacities[1–4]. Modification occurs only if the kitten is exposed selectively during the first three months of life. The peak period of sensitivity for "tuning" seems to occur between days 28 and 35, and thereafter declines rapidly; by the end of the second month, neuronal connections are less readily modifiable[5–6]. The peak of the sensitive period coincides with the clearance of the ocular media[7], the beginnings of adequate locomotor control (M. S. Levine, C. D. Hull, and N. A. Buckwald, unpublished), completion of the most active phase of myelination in the optic nerve and optic tract (C. L. Moore, R. E. Kalil, and W. A. Richards, unpublished), and the period of greatest synaptic density in the visual cortex[8]. In the present study we show that kittens do not spontaneously seek light at this time. Rather, there is a sudden onset of light-seeking behaviour at two months of age, when the cortical contour-coding system is not very sensitive to environmental modification, and many other adult visual characteristics have already been acquired.

Table 1 Pattern of self-induced exposure to visual stimulation in light- and dark-reared kittens

	Short presses (<0.5 s)		Long presses (≥9.5 s)	
	40–49 d	70–79 d	40–49 d	70–79 d
Dark-reared kittens				
1	0.804	0.337	0.000	0.120
2	0.750	0.285	0.000	0.169
3	0.523	0.339	0.000	0.095
4	0.877	0.382	0.000	0.081
5	0.492	0.340	0.000	0.052
Mean	0.689	0.337	0.000	0.103
Light-reared kittens				
1	0.792	0.746	0.000	0.000
2	0.605	0.569	0.009	0.014
Mean	0.699	0.658	0.005	0.007
Auditory control				
Tone	0.500	0.267	0.024	0.000
Light	0.498	0.289	0.043	0.167

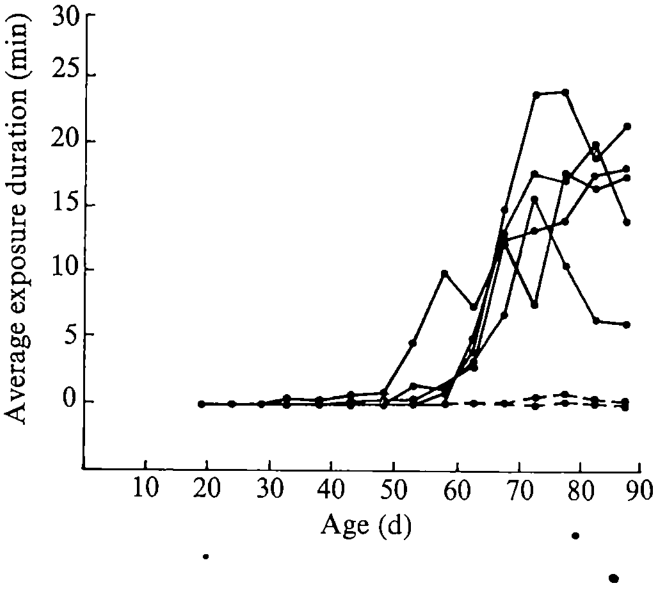
Proportion of short and very long presses within 10-d blocks before and after onset of light seeking.

Beginning at 5 d of age (before the eyes are open), six kittens taken from three litters were housed with their mothers in large nesting cages in a completely dark room where they remained for 3 months. From day 15 for 1 kitten, day 20 for 2 and day 23 for 3 kittens, to day 90, the kittens were transported in a light-tight box and placed individually each day in an experimental chamber, 76 cm wide by 91 cm long. A cylindrical tunnel, 6.5 cm in diameter and 34 cm long, extended from the rear of the chamber. A transparent Plexiglas panel, measuring 10×10 cm and recessed 5 cm, was positioned in the near end of the tunnel. A pattern of stripes subtending a visual angle of 15° was mounted at the far end of the tunnel and was illuminated continuously when the panel was pressed. Different cats were exposed to stripes of different spatial frequencies, but this did not seem to influence the pattern of responding. The tunnel was maintained at eye level by adjusting the level of the floor to compensate for the kitten's growth.

At the beginning of each session the kitten's nose was pressed against the panel several times for a total of about 5 s of stimulation. In addition, after the kitten had been left alone in the chamber, the light was flashed briefly to signal the position of the panel. Subsequently, the kitten could obtain light only by pressing the panel.

Two light-reared controls were treated identically except that they were housed in a larger cat colony maintained on an alternating 12 h light–dark schedule. One of the kittens reared in the dark served as an auditory control. It was allowed access to light only on alternate days. During the sessions every other

Fig. 1 Minutes of self-produced exposure per session, averaged over 5-d periods, expressed as a function of age of the kittens. Solid lines represent individual dark-reared subjects; broken lines, light-reared controls.



day, pressing the panel turned on a tone (maximal energy between 3,000 and 4,000 Hz) instead of the light.

Four of the dark-reared kittens and both light-reared kittens were given two 1-h sessions daily. The auditory control and one other dark-reared kitten were given two sessions per day until day 70 and thereafter were run only once per day.

Figure 1 shows minutes of self-produced exposure per session, averaged over 5-d periods, for all the animals except the auditory control. Neither dark- nor light-reared kittens press for much light in the first two months of life. Beginning at about day 60, however, the dark-reared kittens show a dramatic increase in self-produced exposure to light, in sharp contrast to the controls.

The increase in exposure is accompanied by a change in the pattern of responding. Both the number of presses and the average press duration increase. Before day 60, the typical mean press duration rarely exceeds 1.0 s (median rarely exceeds 0.5 s), but afterwards it seldom falls below 3.0 s (median between 1.0 and 4.0 s). Shorter presses and brief "knocks" become much less frequent, and some very long presses are produced. Before day 60, a press of 5 s or longer is rare, but afterwards presses of 1–3 min are not uncommon. Presses of up to 8 min were recorded on several occasions. Table 1 shows the shift in pattern of pressing for each group. Although there is a marked difference between groups, the pattern is consistent within each group.

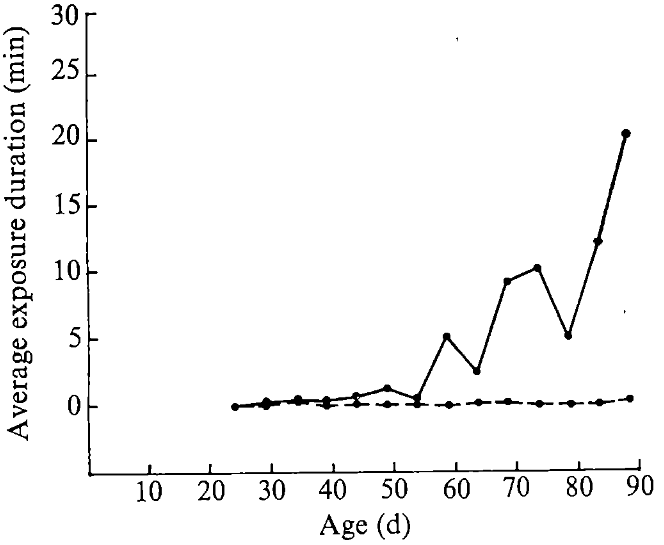


Fig. 2 Minutes of self-produced exposure to light (solid line) or sound (dotted line) per session, average over 5-d periods for one kitten.

All the dark-reared animals begin to change their behaviour at about the same age. Perhaps it could be argued that lack of strength or motor coordination prevents the kittens from panel pressing intensively before two months of age. Our data do not support this notion. All the kittens had pressed the panel for brief periods before day 30. There is no gradual increase in the number of seconds of exposure with time nor is any relationship between onset of the behaviour and body weight apparent.

It seems probable that the kittens were seeking visual stimulation specifically, rather than some general increase in sensory input. Figure 2 shows the data for the kitten allowed alternating access to light and sound. Clearly it does not seek the auditory stimulation. Typically, it would press for several seconds at the beginning of the session and then not press at all for the remainder. When light was available, it would press for prolonged periods. Additional control sessions were run for the other kittens in which, for alternate 15-min blocks within the 1-h session, pressing the panel did not illuminate the pattern. With no light contingency, the kittens quickly stopped responding, even though auditory stimulation was available from a microswitch which operated when the panel was pressed.

As was pointed out, the peak sensitivity for the "tuning" of cortical neurones seems to occur in about the fifth week of life[9–10]. Why should kittens not attempt to gain much access to light until cortical modifiability has declined? Perhaps they only seek such experience when the visual system is approaching a relatively stable stage, i.e., after the ocular media have cleared and the feature detectors have been tuned[6–8].

Our results do not throw light on the functional significance of the onset of light-seeking; the phenomenon does not seem to be related to the development of contour detecting mechanisms, rather it may point to other developmental processes in the visual system. Indeed it has been argued[11] that the main sites at which perceptual learning influences the system are probably not at the contour coding level.

There is a great temporal discrepancy between electrophysiological studies of cortical maturation, which place the peak sensitivity of the critical period at one month of age, and the present behavioural evidence, which demonstrates an abrupt, light-oriented behavioural change about a month later. The present results emphasise that the functional tuning of the contour-coding system of the visual cortex cannot simply be equated with the process of perceptual development.

This research was supported by a grant from the National Research Council of Canada to P. C. D. We thank D. J. K. Mewhort, D. W. Muir and B. J. Frost for helpful comments and criticism.

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Effect of constant light on nychthemeral variations in serum testosterone in male *Macaca radiata*

CIRCADIAN and seasonal variations in reproductive functions of mammals have been investigated extensively[1]. Serum testosterone exhibits a nychthemeral variation in man[2–6] and the rhesus monkey[7,8]. Exposure of laboratory rodents which are nocturnal in habit to continuous illumination causes precocious puberty in immature rats and hamsters[9], and constant oestrus in cycling rats due to elevated gonadotropins[10]. Conversely, prolonged exposure to darkness causes atrophy of testes in the hamster due to suppression of circulating FSH and LH[11,12]. Relatively less information is available on the effects of light on reproduction in the human and non-human primates. From a study of the effects of constant light on serum testosterone in the male bonnet monkey (*Macaca radiata*), we have found that *M. radiata* shows a nychthemeral variation in serum testosterone.

Adult male monkeys weighing 8–10 kg were lodged in individual cages in rooms measuring 10×10×8 feet in our primate facility. Artificial cool white light was provided by installing two fluorescent tube lights (40 W, 250 V) at a height of 10 feet. The rooms were not air-conditioned but were provided with a powerful exhaust fan to maintain good air circulation. Normally, lights are kept on in these rooms between 0600 and 1800 (IST). The animals were kept under this light–dark (LD) schedule of 12 : 12 h for 3 months before being used for experiments. The monkeys were fed pelleted food (Hindustan Lever Monkey food) and vegetables three times a day, water being available at all times.

Blood was withdrawn by venepucture at 6-h intervals. The monkeys were not anaesthetised during these operations. Testosterone in the serum was estimated by radioimmunoassay according to standardised procedures (see footnote for Table 1 for details).

The results show that *M. radiata* like the rhesus monkey (*M. mullata*) also exhibits a nychthemeral variation in the secretion of serum testosterone with a 6–12-fold increase during the night hours (Table 1). Exposure to constant light for 72 h did not bring about significant change in this pattern. Exposure to continuous illumination for 4 weeks, however, abolished the nocturnal surge, this being restored once the lighting was shifted back to normal schedule of LD 12 : 12 (Fig. 1) While restoration to normality seems to be a relatively fast process, a minimum of 15 d of continuous illumination was found necessary to abolish the nocturnal elevation of serum testosterone. In two of the monkeys, although there was a reduction in testosterone levels at 2000 due to constant light, the levels at 0800 and 1400 increased over the control by two- to threefold. The reason for the latter is at present not clear to us.

While in the rhesus monkey no elevation in LH levels have been observed that can be correlated with the nocturnal rise of testosterone[8], in the human Judd *et al*[5] observed that the pulsatile rise of testosterone during the nocturnal period is closely preceded by pulsatile changes in LH levels. But since other workers[13,14] have not observed a change in amplitude and frequency in LH peaks between day and night hours, they suggest that the nocturnal rise of testosterone may be related to a variability of Leydig cell sensitivity to LH during these hours[5].

Table 1 Nychthemeral variations in serum testosterone in male *Macaca radiata*

Time of day	Testosterone equivalent ng per ml serum Mean ± s.e.m. ($n = 7$)
0200	26.0 ± 3.90
0800	3.96 ± 0.69
1400	2.29 ± 0.26
2000	25.6 ± 3.72

The procedure adopted for extraction of steroid from serum and radioimmunoassay was essentially similar to that described by us earlier for progesterone[17]. 2,4,6,7,-^{3}H-testosterone was used as the label and the antiserum used was prepared to testosterone-3-oxime–bovine serum albumin. The antiserum at the dilution used (1 : 40,000 final dilution) cross reacts significantly only with dihydrotestosterone to an extent of 66.0%. The specificity of the antiserum is adequate for the present study, as the latter aims at measuring the total testosterone equivalents.

The nocturnal elevation of testosterone appears from our study not to be influenced by the presence or absence of females in the vicinity, as the males in the present experiment were housed separately from the females. It should, however, be mentioned that the males in the breeding units exhibited a much higher nocturnal level of testosterone (approximately 1.5–2-fold) than the males used in the present experiment.

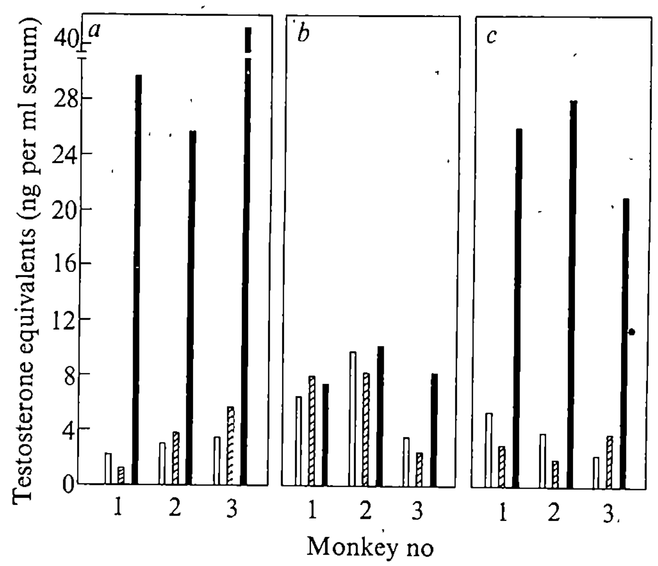

Fig. 1 Effect of continuous illumination on serum testosterone in male bonnet monkeys. Three of the monkeys which showed typical nychthemeral pattern as in Table 1 were selected for this study. *a*, LD, 12 : 12; *b*, LD, 24 : 0; *c*, LD, 12 : 12. Open columns, 0800; hatched columns, 1400; black columns, 2000.

The abolition of nocturnal surge of testosterone in monkeys under constant illumination could be due to either a suppression of gonadotropin secretion or loss of responsiveness of the testis to unchanged levels of gonadotropins.

Interestingly, the monkey differs from the rat in that constant light brings about an increase in pineal hydroxy indole O-methyl transferase (HIOMT) activity resulting in an elevation in melatonin levels[15]. Since it is known that many pineal substances (for example, melatonin) are antigonadotropic in nature, it is possible that an increase of these factors in monkeys under constant light might be responsible for inhibiting testicular function. The observation that blindness in humans is associated with early gonadal maturation[16] lends support to our suggestion. It should, however, be pointed out that as constant light is a stress, a direct action of the factors associated with this stress on the testis cannot be ruled out. All the same it should be mentioned that no change in the time of sleeping was observed when the monkeys were kept under constant light.

In conclusion, the present study shows that the bonnet monkey exhibits a high level of serum testosterone during night hours, which is abolished on exposure to constant light, suggesting that this variation in serum testosterone is a nychthemeral rhythm.

We thank the Indian National Science Academy, New Delhi for financial support. The antiserum to testosterone used in this study was a gift from Dr D. K. Mahajan, The M. S. Hershy Medical Centre, Pennsylvania.

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Received December 2, 1975; accepted March 10, 1976

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Timing of the photoperiod and the hour of birth in rats

THE factors determining the onset of labour are largely unknown, though the regulating mechanisms seem to originate from both the foetus and the mother. The foetus seems to determine the day of birth, through the maturation of the pituitary–adrenal–placental axis, but the mother may provide a fine control, determining the precise hour of birth. Labour varies greatly in its timing and speed of execution, presumably as the result of selection to ensure the maximum probability of survival of both the mother and offspring. Environmental factors such as light, tides and predators are of great importance, providing cues which influence the final hour of birth. Even women, who during childbirth are confined in a most artificial environment, show a marked tendency to deliver in the early hours of the morning[1–4].

The reproductive functions of the laboratory rat are linked to the diurnal changes in light and darkness. When subjected to an artificial photoperiod with light from 0500 to 1900, rats of most strains release their ovulatory surge of luteinising hormone from about 1500 on the day of pro-oestrus, and then ovulate between 0200 and 0400 the next morning[5]. Similarly, at the end of pregnancy, a complex series of changes in the plasma levels of various reproductive hormones[6–12] prepares the animal for labour. As the secretion of these hormones is under hypothalamic control, it may reasonably be asked whether the timing of labour is linked to the photoperiod. The rat gives birth between days 22 and 24 of pregnancy (day 1 = sperm-positive vaginal smear), though reliable data, where all extraneous factors have been excluded, are limited Boer *et al.*[13] recorded by continuous observation an abrupt increase in the incidence of labour at about 1200 on day 22, with a second and much smaller peak during the early hours of day 23. This study involved some, albeit slight, disturbance of the animals, but the rapid onset of births and their bimodal distribution suggested the existence of an accurate timing mechanism. In the results reported here, we have sought to control the various extraneous parameters which might influence labour, to provide reliable information on the hour of normal delivery. We have also investigated the degree to which the time of delivery is determined photoperiodically

The hour of birth was recorded in 446 rats of a Wistar strain (laboratory bred from MRC Porton stock); 296 were primiparous, weighing 240±27 g (s d) at the time of mating; 87 were multiparous, weighing 268±27 g; and the remainder were of unknown parity Groups of 80–120 pro-oestrous rats were caged singly with a male from 1600. The following morning, the males were removed and mating confirmed in about 80% of the females by the presence of copulation plugs on the dropping trays or sperm in the vaginal smear. These animals were transferred to single cages until delivery occurred some 22–23 d later. All other stock were removed from the delivery room and the doors were locked. Sufficient food and water were provided so that replenishment was required only once during pregnancy. An artificial photoperiod of 14 h light : 10 h darkness was provided for the controls and experimental groups until day 12, with the lights on from 0500 to 1900 each day. On day 12 only, the various experimental groups were subjected to a longer or shorter day by the retardation or advancement of the onset of darkness. Thereafter, the ratio of light to darkness remained at 14 : 10; so the rats had effectively been transferred to a new "time zone". The onset of labour was determined by observation every 3 h from 0500 on day 21, the red light of a torch was used during the hours of darkness. This frequency of observation was chosen as a compromise between the need for accurate timing and the need to avoid disturbance. The timing of birth was scored as that 3-h interval within which the first pup was delivered. All dams with litters were removed from the experimental room 6 h after delivery.

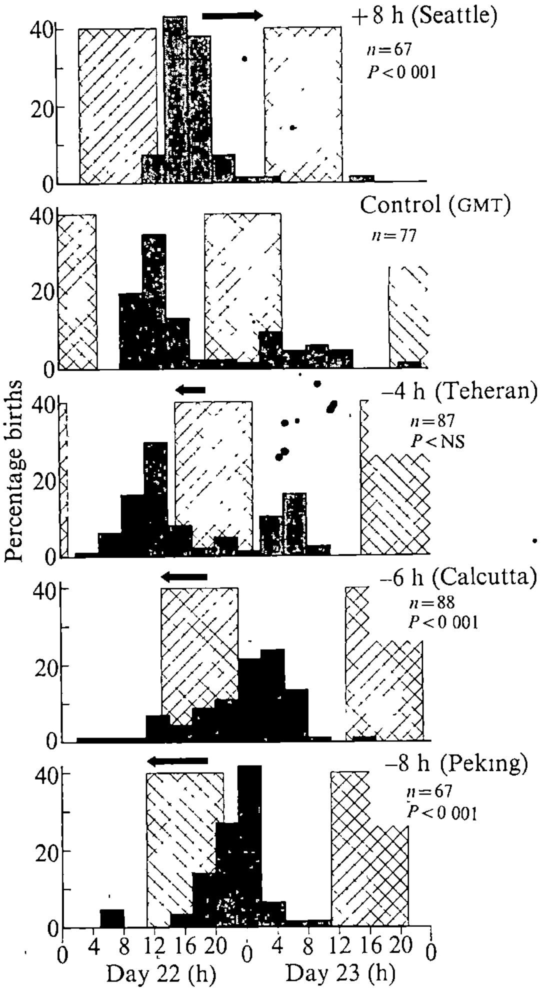

Fig. 1 Effect of the timing of the photoperiod on the hour of birth in rats. The periods of darkness on days 22 and 23 are shown by the hatched areas. The control photoperiod, and the photoperiod of all experimental treatments up to day 12, consisted of 10 h of darkness from 1900 and 14 h of light. Day 12 was lengthened or shortened and the adjusted photoperiod maintained for the rest of pregnancy. The approximate time zones to which, effectively, the rats were transferred are given by reference to the place names. All distributions differ significantly from each other (with the exception of the control (GMT) and −4-h (Teheran) comparison), when compared by either a Kolmogorov–Smirnov or a grouped χ^2 analysis ($P\ll0.001$)

The distribution of deliveries in the control group of 77 rats was bimodal, with mean and median time values of 1619 and 1313 (day 22), respectively (Fig. 1). This pattern of deliveries was very similar to that obtained by Boer *et al.*[13], although the distribution was advanced by about 3 h in all respects. The photoperiod could have been responsible for this time difference, because the animals in Holland[13] were given 12 h of light from 0700 to 1900, but differences due to rat strain and environmental factors cannot be excluded. A further group of animals ($n=55$) was treated identically to the control group except that it was subjected to continuous darkness from the morning of day 21. The timing of labour was almost unchanged; it was again bimodal with mean and median values of 1519 and 1226, respectively. These times were slightly earlier than those of the controls, but the differences were not significant.

Four groups of rats were subjected to time-zone changes in mid-pregnancy; the results are shown in Fig. 1. The lengthening of day 12 by 8 h (the equivalent of transporting the rats from London to Seattle) delayed the time of expected delivery and created a single distribution with mean and median values of 1710 and 1654 respectively. The reduction of day 12 by 4 h (Teheran time) slightly advanced the onset of the distribution (one animal gave birth before 0500). The second peak on day 23 was more accentuated than in the controls, but the overall difference remained non-significant. The reduction of day 12 by both 6 and 8 h (to Calcutta and Peking time) caused profound changes in the distribution. After the 6-h time shift, the mean and median times for delivery were 2322 and 0044 (day 23); whereas, with the 8-h shift, the mean and median values were 2211 and 2306, respectively. Thus the median time of delivery changed by more than 11 h when day 12 was shortened from -4 to -6 h. Delivery seemed to be transferred from the light period of day 22 to the light period of the next day.

The parity of the mother did not influence the hour of birth. The mean litter size was 10.98±2.68 pups (s.d.) for primiparous rats and 10 92±2.78 for multiparous stock, and there was no correlation between the litter size and the hour of birth when the litters contained six or more young (maximum 17). The mortality rate was 4 2% overall, estimated from the number of dead pups 6 h after delivery, but the true figure was probably slightly higher, as some young may have been eaten without trace. Mortality rose to 39% for deliveries after 1100 on day 23, but most of these births were associated with dystocia. Thus the 12 litters that were produced after 1100 were excluded from the calculations of mean and median delivery times.

These results provide clear evidence that the onset of labour in the rat is determined, in part, by the animal's experience of the timing of the photoperiod in the days which precede labour. As in the study of Mitchell and Yochim[14], rats in the present series exhibited a preference to deliver during the hours of light. In spite of the shifting of darkness through the peak of the control distribution, the rats adjusted their time of delivery such that 82% of all births occurred during the 14 h of light Although Svorad and Sáchová[15] claimed that the tendency for the mouse to deliver in darkness could likewise be demonstrated by altering the light regime after mating, Porter[16] found that alterations in the timing of the photoperiod had no effect on the distribution of deliveries, so that mice delivered in light as well as darkness depending on a fixed copulation–delivery interval

In the present experiments, the provision of light *per se* was of no immediate significance, for when the animals were subjected to continuous darkness on the last day the distribution of the deliveries was not retarded; in fact, it was slightly advanced This suggests that it is the biological rhythm of the animal, rather than a cue from the onset of light or darkness on day 21–22, which determines the hour of birth As is known for other biological systems[17], these rhythms take several days to reset when animals are transferred from one time schedule of light and darkness to another, and are not immediately destroyed by the removal of one photoperiodic cue. The daily period of darkness seems to be associated with a physiological change which is inhibitory to labour, but a critical examination of Fig. 1 indicates that this inhibitory effect began to be expressed, as far as deliveries are concerned, 2–3 h before the onset of darkness and it terminated before the lights came on again. Fuchs[18] and Fuchs and Poblete[19] have reported that labour in the rat is presaged by an increase in uterine activity some two or more hours before birth. It is possible therefore that an inhibitory effect on delivery would be influencing maternal physiology 4–6 h before the onset of darkness.

The limits within which normal labour was observed to begin ranged from about 0500 on day 22 to 1100 on day 23, and within this range the median time of delivery varied by up to 12 h according to the timing of the photoperiod. Thus the onset of labour under control lighting occurs relatively early within the normal range, and no photoperiodic change investigated in this study substantially advanced the occurrence of labour The tail of the control distribution, with its second peak on day 23, seems to be a photoperiodic function, that is, some animals are prevented from delivering on day 22 by the impending onset of darkness at 1900 The advancement of day 12 by 8 h may provide a more satisfactory schedule for some experiments, since this resulted in a single statistically normal distribution, with 82% of the animals giving birth between 1400 and 2000 on day 22.

These results raise the important question of whether the endocrinological changes such as the decline in progesterone titres, the increase in oestradiol and prolactin levels, and the augmented output of prostaglandins, all of which are considered to be involved in preparing the uterus for labour, are retarded or advanced by alterations in the photoperiod These changes may, however, proceed on an independent time course, so that the precise timing of labour is determined by the arrival or removal of another and as yet undetermined photoperiod-dependent signal, which permits the activation of the already prepared uterus. Experiments are in progress to examine this question.

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Received January 29; accepted March 10, 1976

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Deleterious effect of an anaesthetic on cultured mammalian embryos

THE teratogenic effect of certain tranquillisers on human and animal pregnancies has been known for many years[1–3]. Anaesthetics and other central nervous system depressants may also be teratogenic when given during early pregnancy, possibly because of the inhibitory action of these agents on mitosis[4]. Anaesthetics can also activate unfertilised mouse eggs *in vivo,* inducing them to develop parthenogenetically[5,6]. Chronic exposure to low levels of anaesthetics may result in a significant increase in spontaneous abortions and the birth of children with congenital abnormalities[7,8]. Experiments with rodents[9] which seemed to demonstrate that chronic exposure to anaesthetics could be teratogenic in early pregnancy have proved difficult to repeat[10], so that doubt has now been cast on the validity of these earlier results. Attention has also been drawn to the possible risk to the human foetus of surgery carried out during pregnancy[11]. In the experiments reported here we have demonstrated a direct, deleterious effect on early postimplantation rat embryos in culture of a single exposure to different doses of an anaesthetic.

Headfold-stage rat embryos of the CFHB strain were explanted during the afternoon of day 10 of gestation (day of finding sperm in the vagina=day 1 of pregnancy), and cultured *in vitro* for 24 or 48 h in heat-inactivated (56 °C, 30 min) homologous IC serum[12], prepared from blood withdrawn from the aorta of rats anaesthetised with ether. During heat inactivation the bottle containing the serum was opened twice to drive off as much ether as possible. Serum from the same batch was used for both control and experimental culture groups, with Avertin (Winthrop) added to the experimental groups. Except where shown in Table 1, the medium was equilibrated with a gas mixture containing 5% O_2, 5% CO_2 and 90% N_2 for the first 24 h and 5% CO_2 in air for the rest of the culture period. These conditions give optimum development *in vitro* for embryos explanted at the headfold stage (D. A. T. New, personal communication). Since the purpose of the cultures was to test the teratogenic effect of a specific anaesthetic agent, the pregnant females were not killed by etherisation but by cervical dislocation. Where embryos from more than one female were used in one experiment, these were divided equally between control and experimental groups.

In preliminary studies the embryos were cultured in watchglasses or 60-ml roller bottles by New's technique[13]. With this equipment the gas phase is considerably greater in volume than the volume of the medium. In this instance no teratogenic effect was obtained even with twice the standard anaesthetic dose of Avertin. In subsequent cultures, sealed embryological watchglasses or 12×75 mm plastic test tubes (Falcon, 2003) containing 3 ml and 5 ml of medium, respectively, were used, such that the volume occupied by the gas phase was less than 0.1 ml and 0.5 ml, respectively.

The standard dose of Avertin used to anaesthetise intact rats was used, where 1 ml of culture medium was assumed to be equivalent to 1 g body weight of an intact rat. The standard dose of Avertin used was 0.02 ml of a freshly prepared 1.2% solution of Avertin dissolved in 0.9% saline per ml of serum (or g body weight of an intact rat), equivalent to approximately 240 p.p.m. (by volume). Further groups of embryos were cultured for 24 h in serum containing twice, four times or six times the standard dose of Avertin. Some of these embryos were examined histologically, while others were transferred with the medium to a 60-ml roller bottle containing 2–4 ml of fresh anaesthetic-free serum and cultured for a further 24 h. At the end of culture, at 24 h or 48 h, embryos were examined under a dissecting microscope and an assessment of development was made using purely morphological criteria, such as the presence or absence of a beating heart, closure of the neural tube, the adoption of the foetal position ('turning'), somite number and the yolk sac diameter. Embryos which were not examined histologically were dissected free from embryonic membranes and their protein content was determined microspectrophotometrically[14].

When embryos were cultured with a large gas phase, there was no difference between controls and those cultured in once or twice the standard anaesthetic dose of Avertin for periods ranging from 10 min to 24 h (the results of this and subsequent cultures are presented in Table 1). When the volume of the gas phase was reduced, embryos grown for 24 h in twice the standard anaesthetic dose clearly developed less well (somites indistinguishable, less embryos with a heartbeat) than controls, and four and six times the standard anaesthetic dose had an even greater effect. When the culture period was extended to 48 h and the protein content of the embryos was estimated, there was a significant difference ($P<0.0025$) between control embryos and those cultured in twice the standard dose of Avertin.

The reduction in volume of the gas phase also resulted in a significant difference ($P<0.0025$) in protein content between control embryos and those grown in the standard

Table 1 Effects on early postimplantation rat embryos of a single exposure to different doses of Avertin

Dose of Avertin (multiples of standard dose)	Exposure to Avertin (h)	Total culture time (h)	Culture conditions	Total no of embryos	Protein content of embryo (μg) mean ± s.e.	Mean yolk sac diameter (mm)	Heartbeat		Average somite no	Neural folds		Turning	
							Present	Absent		Closed	Open	Complete	Partial or none
—	0 (Control)	24*	Static medium large gas phase (60-ml roller bottle)	9	—	2.3	7	2	Not recorded	Not relevant to 24-h cultures			
1	10 min			9	—	2.4	8	1					
1	0.5			9	—	2.5	9	0					
1	1			9	—	1.9	8	1					
—	0 (Control)		Static medium with large gas phase (sealed watchglass)	5	—	2.1	5	0	8				
2	24			5	—	2.2	5	0	9				
—	0 (Control)		Static medium with small gas phase (< 0.1 ml) (sealed embryological watchglass)	5	—	1.2	4	1	7				
2	24			7	—	1.5	4	3	●				
4	24			7	—	1.1	0	7	●				
6	24			7	—	0.9	0	7	●				
—	0 (Control)	48–50	Flowing medium with small gas phase (0.5 ml) for first 24 h (sealed Falcon plastic test tube)	20	187±10	4.0	20	0	23	19	1	18	2
1	24			20	147±7	3.5	20†	0	22	18	2	14	6
2	24			4	64±4	2.9	4‡	0	11	3	1	0	4

●, Indistinguishable.
*5% CO_2 in air for total culture period
†One out of twenty overexpanded pericardia
‡Two out of four overexpanded pericardia.

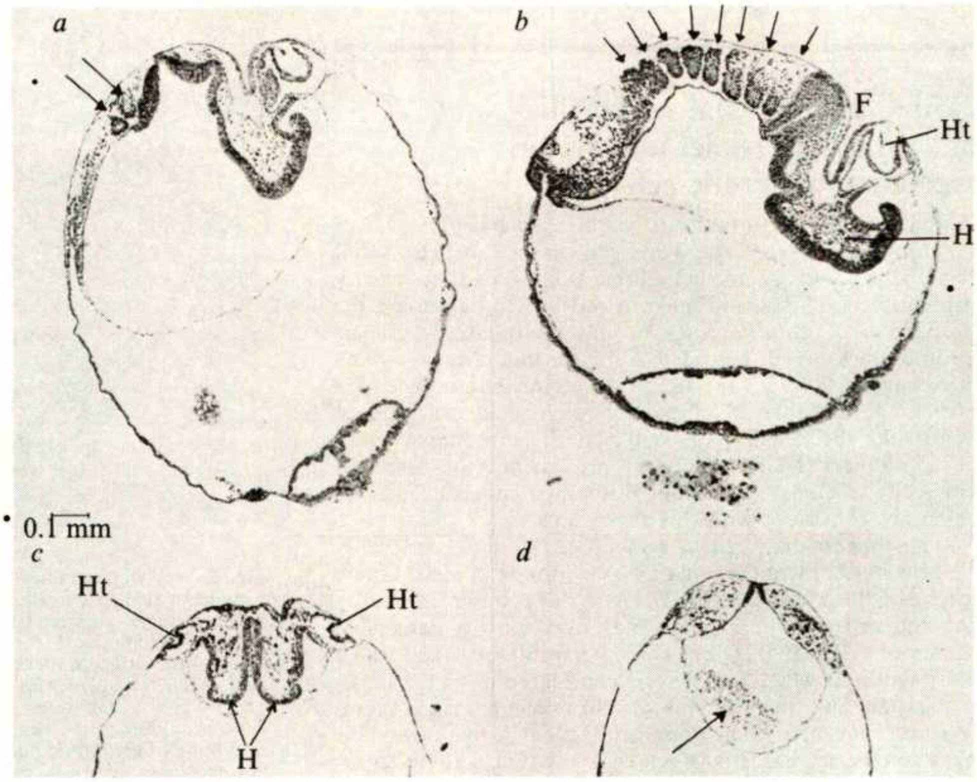

Fig. 1 *a*, Near sagittal section of conceptus cultured for 24 h in twice the standard dose of Avertin. Two poorly formed somites can be seen (arrowed). *b*, Sagittal section of control conceptus cultured for 24 h. Note the larger number of well formed somites (arrowed) compared with (*a*). *c* and *d*, Frontal sections of conceptuses cultured for 24 h in twice the standard dose of Avertin. The unfused heart primordia on either side of the head is shown in (*c*), and the cellular debris (arrowed) found in the amniotic cavity of experimental embryos is shown in (*d*). The scale is same for all figures: bar represents 0.1 mm; F, foregut; H, head; Ht, heart.

dose of Avertin. As in twice the standard dose, all embryos cultured for 48 h developed a heartbeat and closure of the neural folds was unaffected. The frequency of embryos which had 'turned', however, was decreased in the standard dose and twice the standard dose of Avertin compared with the controls. Differentiation, assessed by somite number, was unaffected by the standard dose, but was greatly retarded in twice the standard dose.

Histological examination of control embryos and those cultured in twice the standard dose of Avertin confirmed the detrimental effect of the anaesthetic on differentiation. In the experimental embryos the somites were poorly differentiated and fewer in number (Fig. 1*a*) compared with control embryos (Fig. 1*b*). As well as this effect on the paraxial mesoderm the heart seemed to be affected (Fig. 1*c*). In four out of seven embryos cultured in twice the standard dose of Avertin, cell debris was present in the amniotic cavity (Fig. 1*d*). This was also found when embryos were cultured in four and six times the standard dose. In the latter group the embryonic material consisted almost entirely of dead or dying cells. Debris was not found in the amniotic cavity of control embryos.

These experiments have demonstrated that the anaesthetic Avertin can be teratogenic in the rat, and that the degree of embryotoxicity is dose dependent. When a range of parameters of development was examined, rat embryos which had been cultured in serum containing Avertin were retarded compared with controls. It is not clear whether the type of growth retardation which was observed in apparently normally differentiating embryos as a result of treatment with standard dose of Avertin could be corrected by compensatory growth later in gestation[13].

The main advantage of the model used here over, for example, cell lines in tissue culture as a teratological test system, is that the use of whole embryos more closely resembles the situation *in vivo*. There is, however, the important difference that all the observations reported here result from the direct effect of known doses of Avertin on the embryo, independent of secondary maternal factors such as placental permeability. Further, the anaesthetic is metabolised *in vivo*, and the metabolic products may be more or less teratogenic than the original agent.

In view of these positive results, the factors which remain to be examined include the establishment of the minimal teratogenic dose and exposure duration of a range of anaesthetics. Experiments should also be carried out with embryos of different gestational ages, and different species. It would also be interesting to compare the sensitiveness of our experimental model with other models now in use for teratological testing.

It seems likely that anaesthetics other than Avertin may be teratogenic in species other than the rat. It would therefore be of clinical importance to determine whether any of the anaesthetic agents commonly used in human practice have a similar embryotoxic action when administered during early pregnancy.

We thank Dr D. A. T. New for advice and discussion, and Mrs S. M. Jackson for technical assistance. This work was supported by a grant from the Ford Foundation. C.E.S. is supported by an MRC grant to Dr G. M. Morris.

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Received January 13; accepted March 3, 1976.

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Partial chromosomal set labelling induced by ultraviolet microbeam irradiation of mitotic cells

It would be useful to find out whether a particular part of the chromosome set in the living cell can be labelled so that the labelled and unlabelled chromosomes can be clearly distinguished. This would make it possible to determine the localisation of chromosomes during interphase when the chromosomes are dispersed as tangled thin threads which cannot be traced with the light or electron microscope. We have suggested[1] that an ultraviolet microbeam can be used to provoke the labelling of part of a chromosome set with ^{3}H-thymidine (^{3}H-TdR). Here we present data on mammalian cell labelling following ultraviolet microirradiation which are consistent with this suggestion.

When mammalian cells are incubated with ^{3}H-TdR only the cells normally undergoing DNA synthesis (S phase) show uptake of the tracer into the DNA. After ultraviolet irradiation the cells not in S phase may also show a detectable uptake of ^{3}H-TdR (ref. 2). This is a result of unscheduled DNA synthesis which is generally considered to be the result of excision and replacement of ultraviolet-damaged bases by repair enzymes[3]. The unscheduled DNA synthesis also takes place after a partial nuclear irradiation with an ultraviolet microbeam. Ultraviolet microirradiation produces an alteration in an irradiated volume of nucleoplasm without apparently affecting the remainder of the nucleus or the surrounding cytoplasm[4]. It was found that ultraviolet microirradiation of the nucleus of HeLa or KB cells not in S phase produced a localised ^{3}H-TdR incorporation only over the zone actually irradiated by ultraviolet light[4–6]. Such observations of partial nuclear labelling have been obtained in experiments with interphase cells. During mitosis the chromosomes condense into clearly defined thick strands and the microbeam can be focused on to a distinct part of the chromosome set or even to a small region of one chromosome[7]. We have proposed that DNA of the microirradiated mitotic chromosomes can also be selectively labelled with ^{3}H-TdR and the postmitotic localisation of the labelled DNA can be determined by autoradiography.

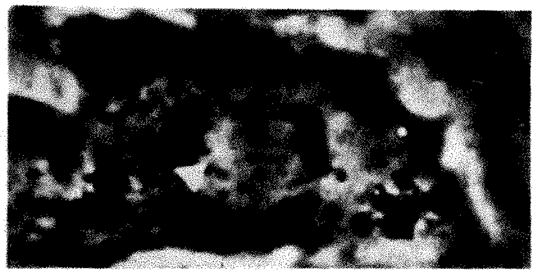

Fig. 1 Typical anaphase SPEV cell during ultraviolet microirradiation. The light triangle over the left chromosomal group indicates the area of irradiation (exposure 5 s at a dose rate of ~2×10^{-3} erg μm^{-2} s^{-1}). Phase contrast ×1,500.

The experiments were carried out on pig embryo kidney cells (SPEV) routinely maintained in medium 199 supplemented with 10% foetal calf serum. The cells were used 24 h after they were seeded in monolayer on a quartz coverslip mounted in a window of a sterile culture chamber. These cells were chosen because they remain relatively flat during the mitotic process, thus making the chromosome set easy to irradiate. The cells were irradiated through the coverslip by an inverted monochromatic (280 nm) ultraviolet microbeam with the spot area about 4 μm^2. A suitable dose was determined empirically by varying the time of irradiation. During metaphase or anaphase 20–25% of the chromosome set of each cell was irradiated. The chromosomal target was always located near the edge of the metaphase plate or within one group of anaphase chromosomes. The pattern of the anaphase cell irradiation is shown in Fig. 1. After irradiation of the anaphase cell only one of the daughter cells obtained a portion of irradiated chromosomes, but the other one could be used as a control. Incorporation of ^{3}H-TdR into postmitotic nuclei was studied by autoradiography.

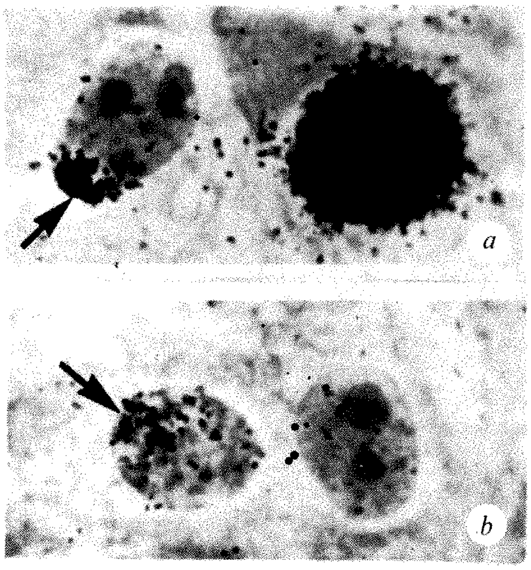

Fig. 2 Autoradiographs of interphase SPEV cells. The cells were incubated in culture medium containing ^{3}H-TdR (10 μCi ml^{-1}). Incubations were carried out for 1.5 h immediately after partial irradiations of anaphase chromosomes. The cells were then rinsed with culture medium and fixed in 3:1 ethanol–acetic acid. After treatment with 3% perchloric acid, autoradiographs using M emulsion were prepared and developed after 10 d exposure. Preparations were stained with haematoxylin. *a* and *b* (left hand), Postmitotic nuclei containing a portion of irradiated chromosomes. Arrows indicate the "patches" of unscheduled ^{3}H-TdR labelling. *a* (right hand), Non-irradiated nucleus in normal S phase. *b* (right hand), Unlabelled control nucleus not in S phase, ×1,500.

Fifty-six irradiated cells were studied. Within 1.5–2 h of irradiation these cells had completed cell division and formed daughter nuclei with decondensed chromosomes. These nuclei contained apparently normal interphase nucleoli, but sometimes they also included some prenucleolar bodies. The appearance of these bodies may be due to nucleolar organiser damage by ultraviolet microirradiation[8,9].

Autoradiography showed that partial ultraviolet irradiation of the mitotic chromosomes provoked unscheduled DNA synthesis in all cases. This synthesis can be clearly detected in the daughter nuclei as patches of unscheduled incorporation of ^{3}H-TdR. These labelled patches are presented in Fig. 2*a* and *b*. The label was never seen in the nuclei of the control daughter cells, since all these cells were not in S phase. The average grain number in the labelled patches (28 grains) was about 20 times greater than that in control nuclei. In the 22 nuclei studied the labelled patches occupied no more than 30% of the nucleus area (Fig. 2*a*). The remaining 34 nuclei exhibited incorporation of ^{3}H-TdR in 30–50% of the nucleus area (as shown in Fig. 2*b*). It is extremely likely that these patches of labelling are situated over the sites of the postmitotic localisation of the irradiated chromosomes.

Our results provide the first demonstration of localised DNA synthesis in interphase nuclei following ultraviolet microirradiation of mitotic chromosomes. Further studies are in progress with the aim of using this technique in an attempt to elucidate the localisation of different parts of the chromosome set during interphase. These studies should take into account the possible influence of irradiation on the postmitotic arrangement of chromosomes. Further experiments should, however, be carried out on cells with chromosomes which are few in number and large in size.

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Received January 30; accepted February 24, 1976.

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Mutation frequency at a recessive locus in haploid and diploid strains of a slime mould

IT is puzzling that mutants are isolated at a similar frequency in tissue cultures of haploid and diploid strains[1–3], for twice as many mutations have often been assumed necessary to produce a mutant diploid as to produce a mutant haploid. Explanations for the apparent high frequency of mutation in diploids[1–6] include epigenetic effects[1–3], changes in gene dose[7], dominant mutations[8], recessive X-linked mutations[4] and heterozygosity before selection[9]. Most recessive autosomal mutants are assumed to arise because of haploidy for the chromosome containing the mutant locus, since most cell cultures are aneuploid[4,10,11]. It is difficult to measure the mutation frequency at recessive loci in cultured haploid and diploid strains because of the paucity of haploid strains and the problems caused by aneuploidy. The slime mould *Dictyostelium discoideum*, a simple eukaryote, is suitable for this purpose, however, as both haploid and diploid strains are available[12–14] and aneuploids are so unstable or inviable that none has been isolated[14]. Using this organism, I have found, contrary to the expectation that mutant diploids should be extremely rare (say 2×10^{-12} diploid, 10^{-6} haploid)[1–6], that the difference in mutation frequency between haploids and diploids for one recessive locus is only an order of magnitude.

Isogenic haploid (NP73) and diploid (DP62) strains of *D. discoideum* able to grow in axenic medium and derived from strain V12 (K.L.W., in preparation) were used. Strains NP73 and DP62 were isolated from the same culture, and strain DP62 is a diploid, formed presumably by the parasexual fusion of two haploid amoebae of strain NP73. The fruiting bodies of both strains have a similar appearance when amoebae grown on slime mould (SM) agar plates with *Aerobacter aerogenes* are allowed to sporulate, although growth of DP62 is slightly slower and the spore masses are shorter than NP73. All 28 haploid segregants of strain DP62, obtained by prolonged growth of DP62 in axenic medium[14] or as faster growing sectors on agar plates, were indistinguishable from strain NP73. Both strains carry the spore shape marker *sprB2* (ref. 17) which makes the distinction between haploid and diploid strains very easy (Fig. 1*a* and *b*). To confirm the ploidy, both strains were grown axenically and their chromosomes were stained; NP73 has seven chromosomes and DP62 has 14 (Fig. 1*c* and *d*).

Table 1 Spontaneous frequency of occurrence of methanol resistance (*acrA*) in isogenic haploid and diploid strains of *D. discoideum*

Strain	No. of mutants*	Frequency ± s.e.
Haploid NP73	115	$7.2\pm1.6\times10^{-7}$
Diploid DP62	15†	$8.8\pm4.6\times10^{-8}$

The table shows the combined results from the examination of 1.6×10^{8} amoebae of strains NP73 and DP62. For each of eight clones, 10 methanol-containing (2% v/v) SM agar plates[17] were inoculated with 2×10^{6} amoebae and a heavy inoculum of the bacterial associate *Aerobacter aerogenes*. We had established that the frequency of occurrence of *acrA* mutants is independent of the number of amoebae plated over a wide range of inoculation densities (at least 10^{5}–2×10^{6} per plate)[17]. Resistant colonies were isolated from the methanol-containing SM agar plates after 7 d at 22 °C; no additional resistant colonies were observed at day 14. All resistant colonies were passaged on SM agar and retested on SM agar containing methanol to confirm that resistance was maintained in the absence of methanol. The plating efficiency on SM agar containing methanol (2% v/v) of two haploid and two diploid methanol-resistant strains studied further was 100%. The ploidy of all mutants was assessed by spore size and shape. The ploidy of several methanol-resistant haploid and diploid strains was confirmed by chromosome staining (Fig. 1).

*Haploids, *acrA*; diploids, *acrA*/*acrA*.

†Includes one haploid strain which is excluded in calculating the frequency.

The *acrA* locus located on linkage group II was studied because it is the only site of methanol resistance in *D. discoideum*[15,16]. The phenotype of strains with a mutation at the *acrA* locus is recessive resistance to acriflavin (100 μg ml^{-1}), methanol (2% v/v) and thiabendazole (10 μg ml^{-1})[15]. Isogenic haploid (NP73) and diploid (DP62) strains were plated on to SM agar plates containing methanol (2% v/v)[15] to obtain mutants at the *acrA* locus.

Table 1 summarises the results of experiments where 2×10^{7} amoebae of each of eight different clones of strains NP73 and DP62 were plated on SM agar plates containing 2% methanol. All experiments with haploids and diploids were conducted in identical conditions using the same batches of methanol-containing plates. All resistant mutants shown in Table 1 maintained resistance to methanol when passaged in the absence of methanol. Ploidy was determined according to spore size and shape, and in some cases chromosome numbers (Fig. 1).

Spontaneous mutations to methanol resistance in haploid strain NP73 occurred at a frequency of 7.2×10^{-7}; this is within the range reported for other haploid strains of *D. discoideum* (10^{-7}–2×10^{-6}, refs 15–18). Spontaneous methanol-resistant diploids were isolated from strain DP62 at a frequency of 8.8×10^{-8}; this was approximately eight times less than the frequency in the haploid strain. A single haploid, methanol-resistant strain was obtained from strain DP62 (Table 1). This strain probably arose either by early haploidisation of a methanol-resistant diploid, or by mutation in a haploid amoeba in strain DP62. All diploid strains of *D. discoideum* contain haploids at a low frequency, usually $<10^{-3}$.

If methanol resistance in the diploids was due to homozygosis for a recessive mutation, all haploid segregants from such a diploid would be resistant to methanol. On the other hand, if resistance arose due to a dominant mutation, only half of the haploid segregants should be resistant to methanol. To rule out the possibility of dominant alleles at the *acrA* locus, 15 independently derived haploid segregants isolated as faster growing sectors from a methanol-resistant diploid on SM agar plates were screened for methanol resistance. All were resistant, confirming observations that mutations at the *acrA* locus are recessive[15–18].

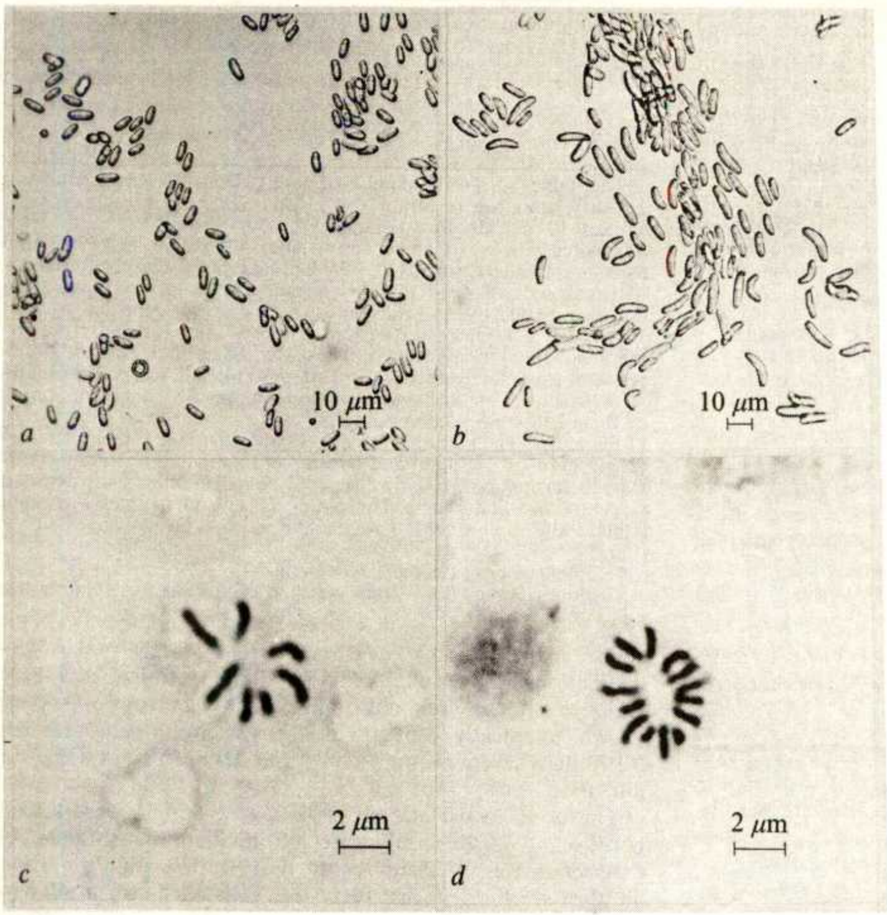

Fig. 1 *a*, Spores of haploid strain NP73 (× 300). *b*, Spores of diploid strain DP62 (× 300); the diploid spores are slightly boomerang-shaped and are considerably longer than the haploids. *c*, Late prophase of haploid strain NP73 showing seven chromosomes (× 3,250). *d*, Late prophase of diploid strain DP62 showing 14 chromosomes (× 3,250). Both strains were grown axenically and the chromosomes were fixed and stained with Giemsa stain as described previously[14].

Studies of diploid seeds of the tomato *Lycopersicon esculentum* have shown that, when the mutagen hydrazine is used, the frequency of mutants homozygous at specific recessive loci is similar to that of mutants heterozygous at the same loci[19]. Together with these results, my studies of *D. discoideum* show that the unexpectedly high frequency of mutants homozygous at recessive loci in diploids is not a unique property of cell tissue culture systems; it may even be a general property of diploid cells. An attractive hypothesis which may explain the observed high frequency of mutants at recessive loci in diploids is that they arise by a single mutation followed by recombination, for example, mitotic crossing over or gene conversion, rather than by two independent mutagenic events[6,11].

Tests of this hypothesis are in progress, using a methanol-sensitive diploid strain of *D. discoideum* with markers flanking the *acrA* locus–linkage group II, centromere, *whiA*/+, +/+, *tsgD*/+ (ref. 17). The recessive, growth-temperature-sensitive marker *tsgD* is further from the centromere than *acrA*. If methanol-resistant diploids arise by a single mutation followed by mitotic crossing over, then 50% of methanol-resistant diploids would be temperature sensitive for growth, *tsgD*/*tsgD*, provided that there is an equal probability of either homologue being mutated. Examination of 20 independently derived methanol-resistant diploids provided some support for this hypothesis, for six were growth-temperature-sensitive and 14 grew at the restrictive temperature. There are however, some problems. The high mutation frequency ($\sim 10^{-7}$) does not merely result from sequential mutation and mitotic crossing over without interaction between these processes, since the expected frequency on this basis would be approximately 10^{-10}, that is $\sim 10^{-6}$ (mutation frequency, refs 15 and 17) $\times \sim 10^{-4}$ (frequency of mitotic crossing over between the centromere and *acrA*)[15,17].

I thank Gillian Robson for technical assistance, and Richard Kessin, Peter Newell and members of the Genetics Department, Australian National University, for criticisms.

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Received December 18, 1975; accepted March 10, 1976.

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Metabolic cooperation between human fibroblasts with normal and with mutant superactive phosphoribosylpyrophosphate synthetase

METABOLIC cooperation is a form of intercellular communication by which cells in contact exchange molecules, a process providing multicellular organisms with an important mechanism for control of metabolic activity[1]. Subak-Sharpe *et al.*[2] observed contact-dependent transfer of purine nucleotides from normal cells to cells mutationally incapable of producing inosinic acid due to deficiency in hypoxanthine–guanine phosphoribosyltransferase[3]. Metabolic cooperation of this type was later also demonstrated with other enzymic markers, such as adenine phosphoribosyltransferase and thymidine kinase[4,5]. Such transfers are characterised by the normal cell being the donor and the mutant cell being the recipient, the former transferring to the latter a mutationally lacking metabolite. We report here on a new form of contact-dependent metabolic cooperation, unique in that the transfer of a metabolite occurs from a mutant donor cell to a normal recipient cell.

We were able to demonstrate this phenomenon for cultured human fibroblasts using a new enzymic marker, a mutant feedback-resistant superactive phosphoribosylpyrophosphate (PRPP) synthetase. The principle of transfer along a gradient is, however, maintained in that the donor mutant cell is metabolically more active than the recipient normal cell. This marker has been found to be the primary abnormality underlying excessive purine production in a family affected with primary metabolic gout[6,7]. The mutation is manifest in superactivity of the enzyme in the physiological cellular milieu, being due to decreased sensitivity of the mutant enzyme to feedback inhibition by several physiological intracellular inhibitors such as ADP, GDP and 2′,3-diphosphoglyceric acid. This superactivity increases availability of its reaction product PRPP, a key substrate for both *de novo* and salvage pathways of purine nucleotide synthesis. The consequent increased salvage capacity thus enables selection between normal and mutant cells, and the excessive *de novo* purine synthesis enables determination of the proportion of mutant cells in a culture containing a mixture of mutant and normal cells.

The fibroblast growth medium was modified[8] to allow survival of only the mutant cells, that is, cells possessing increased capacity to produce purine nucleotides. 6-Methylmercaptopurine riboside (6-MMP-riboside; 0.2 mM) was added to block *de novo* purine synthesis[9], and hypoxanthine (0.2 mM) and uridine (0.5 mM) were added to enable salvage synthesis of inosinic acid and uridylic acid, respectively. Experiments were designed to determine whether the capacity to resist selection in these conditions could be transferred from mutant to normal cells (for details, see Table 1). Normal and mutant cells were mixed in ratios of 1:1 and 1:3, respectively, and cocultured for 3–10 generations. Table 1 shows that the capacity of mutant cells to resist selection could be transferred to the normal cells, the degree of transfer depending on the degree of contact between normal and mutant cells during selection—the greater the proportion of mutant cells in the cell mixture and the greater the density of the cells in culture during selection, the greater the probability of contact between the mutant and normal cells. After exposure to selection of a normal–mutant cell mixture (1:1) at high cell density, the rate of *de novo* purine synthesis was similar to that found before selection, being the average of the synthesis rates of separate normal and mutant cell cultures. On the other hand, when the same mixed cultures (1:1) were exposed to selection at low cell density, the rate of purine synthesis after selection increased to approximately twofold, indicating survival of the mutant cells only. The acquired resistance of the normal cells against selection was markedly reduced when normal cells were mixed with mutant ones at a 3:1 ratio. After exposure to selection of such a cell mixture at low cell density, the surviving cells were too few to allow propagation to confluency, indicating the death of most of the normal cells. When the same 3:1 cultures were exposed to selection at high cell density, however, there were enough survivors to allow propagation, and the rate of *de novo* purine synthesis in the culture was increased to 2.5-fold compared with the preselection value. These results indicate that in this latter experiment a small proportion of the normal cells survived the selective conditions, because, if all normal cells have been killed, the rate of purine synthesis should have increased approximately to fourfold.

We are assuming that the increase in the rate of purine synthesis reflected death of the normal cells. That this was indeed the case, and that there was no increase in the rate of *de novo* purine synthesis in mutant cells, is evident from experiments in which mutant cell cultures were similarly exposed to the selective conditions. In such mutant cells the rates of purine synthesis before and after selection were found to be the same

That this metabolic cooperation was contact dependent was determined as follows. One coverglass covered with normal fibroblasts and two coverglasses covered with mutant fibroblasts were placed side by side without physical contact

Table 1 Rate of *de novo* purine synthesis in mixed normal–mutant fibroblast cultures after selection against normal cells

Source of cells	Rate of incorporation of ^{14}C-formate into purines excreted by cells into incubation medium (c.p.m. per mg protein per 6 h)		
	Before selection	After selection using:	
		Dense cultures	Dilute cultures
Normal subjects			
J.B.	8,120	All cells killed	
J.Be	12,050	All cells killed	
Subject O G. with mutant enzyme	89,293	106,301	86,668
Mixture of normal and mutant cells			
O.G.+J.B. 1:1	59,294	70,447	94,567
O.G.+J.Be 1:1	58,717	61,163	90,116
O.G.+J.Be 1:3	27,269	66,246	Too few survivors to allow propagation

Biopsies (3 mm) were removed from the forearm skin, minced, placed in Falcon Plastics sterile disposable bottles and flooded with Eagle's MEM containing Earle's Balanced Salt Solution (Gibco, F–15), 15% foetal calf serum, penicillin (100 U ml^{-1}), streptomycin (100 µg ml^{-1}) and mycostatin (25 µg ml^{-1}). Media were saturated with a 5% CO_2–95% air environment, and the bottles stoppered and incubated at 37 °C until outgrowth was apparent. Cells were subcultured and serially propagated until confluency. Cell mixtures which were prepared after trypsinisation, were cocultured for 2–3 generations. Homogeneous and mixed cultures were exposed to selection at high (25,000 cells cm^{-2}) and low (1,000 cells cm^{-2}) cell densities, by addition to the regular culture medium of 0.2 mM 6-MMP-riboside, 0.2 mM hypoxanthine and 0.5 mM uridine. The selection medium and regular medium were changed every second day. Selection was carried out for one week, after which the cultures were flooded with the regular medium and propagated until confluency Cells were collected by trypsinisation and divided into two Falcon flasks and propagated until half confluency was obtained ($0.5–1.5 \times 10^6$ cells per 75–cm^2 Falcon Plastics culture flask), at which stage the cultures were studied for the rate of *de novo* purine synthesis by measuring the incorporation of ^{14}C-formate into total purines excreted by the intact cells into the incubation medium, essentially as described previously[7,8]. The non-confluent monolayer cultures were incubated for 6 h with 10 ml fresh growth medium containing 10 µCi ^{14}C-formate. At the end of incubation, the total volume of the incubation medium, after removal of cells by centrifugation, was transferred to tubes and acidified with 2.0 ml 6 N perchloric acid; 0.2 µCi 8-^{3}H-adenine were added and the tubes thoroughly mixed and maintained at 100 °C for 1 h The hydrolysate was centrifuged and the supernatant adjusted with water to 15 ml and chilled. To each tube 7 mg adenine was added as carrier, the purine bases precipitated as purine silver complex, washed, extracted with 0.1 N HCl at 100 °C, and the radioactivity was counted Results were corrected for incomplete recovery of purines, using 8-^{3}H-adenine as reference, and related to total cell protein. For all fibroblasts, labelling of purines excreted into the medium was linear with time for at least 8 h, and, for a fixed period of incubation, was also proportional to the number of cells incubated.

in one Petri dish, and flooded with selective medium. The normal cells started to degenerate within 24 h, all dying within 1 week, whereas the mutant cells remained alive without signs of degeneration.

No attempt has been made as yet to identify the molecule, the contact-dependent transfer of which is responsible for the metabolic cooperation. Four possibilities may be considered: transfer of inosinic acid or other purine nucleotides, transfer of the enzyme PRPP synthetase or of its reaction product PRPP, or transfer of molecules containing the information for the synthesis of the mutant superactive PRPP synthetase, such as DNA or RNA. That the transferable molecule has a short lifespan in the recipient cell is indicated by the dependence of metabolic cooperation on the presence, during selection, of close contact between the mutant and the normal cells, and by the observation that prolonged contact between normal and mutant cells before exposure to selective medium does not render normal cells resistant to selection. In view of what is known about contact-dependent metabolic cooperation[1–5], the short lifespan may indicate that this molecule is either the reaction product, PRPP, or purine nucleotides. That nucleotides are transferred has already been demonstrated in other cases of contact-dependent metabolic cooperation; whether PRPP is also transferred has not yet been clarified. Nevertheless, a transfer of PRPP is likely in view of its higher concentration in the donor cell and its small molecular weight.

Supported by the US–Israel Binational Science Foundation.

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Altered glucose-6-phosphate dehydrogenase in bromodeoxyuridine-substituted cells

IT has been adequately demonstrated that BUdR is mutagenic in bacteriophage[1,2,3] when incorporated in place of thymidine in DNA, whereas a clear demonstration that it is mutagenic in eukaryotic cells is still lacking. We have described the selection of two variant hamster cell lines that survive with extensive levels of BUdR substitution in DNA[4,5] and that have been maintained in continuous cultivation for more than 300 generations without loss of viability. We have found no evidence of alterations in the nucleotide ratio of nuclear DNA consistent with the accumulation of BUdR-induced base transition on long term cultivation of the cells, and analysis of rRNA made in the substituted cells offers no evidence that BUdR causes base transitions within the genome or transcriptional errors which result in the accumulation of faulty RNA transcripts[6].

As a more sensitive test to detect BUdR-induced alterations in the genome or in transcription products, we have begun to examine various enzymes produced in the parent and substituted cell lines. The heat lability of an enzyme is frequently used as one criterion of an altered primary sequence of that protein. Thus, senescent WI-38 and MRC-5 cell cultures have increased fractions of heat labile glucose-6-phosphate dehydrogenase (G6PD)[7] and lactic dehydrogenase[8]; ageing mice have an increased number of inactive liver aldolase molecules[9], and cultured fibroblasts taken from patients with either progeria[10] or Werner's

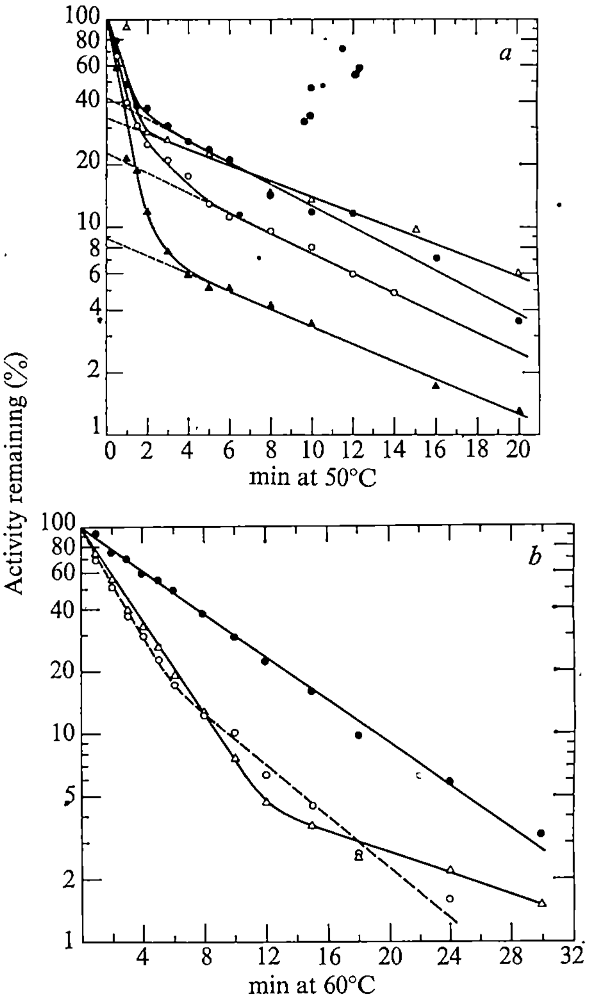

Fig. 1 *a*, Heat inactivation of G6PD from BUdR-substituted cells. Confluent roller bottles were rinsed twice with 250 mM sucrose, 1 mM EDTA, and 1 mM 2-mercaptoethanol, *p*H 8.2, and the cells were scraped off in the same solution. Subsequent steps were carried out at 4 °C. Cells were pelletted at 1,500 r.p.m. for 10 min, resuspended in the sucrose solution at approximately 10^7 cells per ml and sonicated by three bursts of 10 s each at 7 mA in a Bronwill sonicator (microprobe). Debris was removed by centrifugation at 600*g* for 10 min, and the supernatant then spun at 100,000*g* for 1 h Protein was determined by the method of Lowry *et al.*[21], and all samples were adjusted to approximately 1.5 mg protein per ml and stored in liquid nitrogen. Assays for G6PD activity contained: 50 mM Tris-HCl, *p*H 7.5, 10 mM $MgCl_2$, 1 mM NADP and 2 mM G6P. The final volume was 1 ml, containing from 0.025 to 0.2 ml of enzyme. Enzyme samples were heated at 50 °C for the appropriate time, quenched on ice and transferred to the complete reaction mixture at 25 °C. Assays were carried out at 25 °C in a Gilford 2400 spectrophotometer, following the initial rate of increase in absorbance at 340 nm due to the reduction of NADP. Activity in the unheated enzyme was dependent on the addition of both G6P and NADP. ●, 3460; ○, B4; ▲, HAB; △, a mixture of 3460 and HAB with relative G6PD activities of 2:1. *b*, Heat inactivation of G6PD isolated in the presence of 1 mM NADP. Enzyme samples were prepared and assayed as in (*a*) except that the enzyme was always in the presence of 1 mM NADP and heated at 60 °C. ●, 3460; ○, B4; △, HAB.

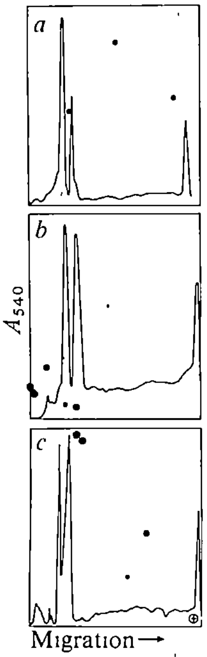

Fig. 2 Electrophoresis of G6PD on polyacrylamide gels. Samples were electrophoresed for 4 h and 3 mA per gel on 11-cm 8% polyacrylamide disk gels according to Bakay and Nyhan[21]. The running buffer was 5 mM Tris-HCl, *p*H 8 1, and 40 mM glycine. Bromophenol blue was added as a marker and ran to about 1 cm from the end of the gel at the termination of electrophoresis. Gels were stained for G6PD activity for 45 min at 37 °C in a solution containing: 100 mM Tris-HCl, *p*H 7.6, 10 mM $MgCl_2$, 0.5 mM NADP, 3.5 mM G6PD, 0.73 mM nitroblue tetrazolium, and 0.2 mM phenazine methosulphate. Gels were then fixed in 7% acetic acid and scanned at 1 cm per min for G6PD activity at 540 nm in a Gilford 2400 spectrophotometer fitted with a linear transport unit. Electrophoresis is from left to right, with the bromophenol blue marker at the extreme right. Peaks of G6PD activity are designated I and II corresponding to the most slowly and rapidly migrating, respectively. *a*, 3460; *b*, B4; *c*, HAB.

syndrome[11,12], both of which show clinical manifestations of premature ageing, have an increased fraction of heat labile G6PD, 6-phosphogluconate dehydrogenase and hypoxanthine-guanine phosphoribosyl transferase.

If BUdR is acting as a mutagen in the substituted hamster cell lines, we might expect to find differences in the heat lability and/or other enzymatic properties of some enzymes which could be the consequence of alterations in the primary sequence of proteins due to BUdR-induced base transitions during replication or transcription We report here that both *in vitro* and *in vivo* experiments have shown that substituted cells contain an altered population of G6PD molecules, which correlates well with the level of BUdR substitution in the genome.

The cell lines used have been described before[4,5]. Briefly, 3460 cells are derived from a Syrian hamster melanoma (RPMI 3460), are grown in Dulbecco's modified Eagle's medium in the absence of BUdR and routinely have a cloning efficiency of >95%. B4 cells are derived from 3460, are grown in the presence of 10^{-4} M BUdR (required for optimum growth) and have a cloning efficiency of ~90%; the nuclear DNA is 62% substituted with BUdR. HAB cells are derived from B4 by growth in media containing 0 1 mM hypoxanthine, 0.4 μM aminopterin, and 10^{-5} M BUdR; the cloning efficiency is routinely ~85% and the nuclear DNA is ⩾ 99.8% substituted with BUdR. The generation times are approximately 16, 28 and 36 h for 3460, B4 and HAB cells, respectively. Both B4 and HAB cells have undergone more than 300 population doublings without apparent loss of viability and had undergone more than 175 population doublings at the time of our experiments.

Human erythrocyte G6PD consists of a single polypeptide chain encoded for by an X-linked gene[13], and is active in both dimeric and tetrameric forms, but inactive as a monomer[14]. This is probably true of rodent cells as well[15], although the enzyme activity may be partially regulated by autosomal loci[16]. For heat denaturation studies crude enzyme preparations were made from 3460, B4 and HAB cells (in the absence of NADP). Their heat lability is shown in Fig 1*a*. The G6PD activity from all three cell lines shows a biphasic denaturation, with both a rapidly and a slowly denaturing component. When extrapolating the linear portion of the curve (slowly denaturing) to the ordinate, we calculate that the fraction of heat labile G6PD molecules in 3460, B4 and HAB cells is 59%, 77% and 91%, respectively The average fraction of heat labile G6PD molecules from four different preparations is 56.5%, 69.7%, and 79 7% for 3460, B4 and HAB cells, respectively (Table 1) This difference is not due to different protein concentrations of heated enzyme preparations since they were adjusted to nearly equal protein concentrations before heating and assay. More important, dilutions of each enzyme preparation gave the same value for the fraction of heat labile molecules on extrapolation, although the rate of decay varied with protein concentration (data not shown). It is unlikely that endogenous inhibitors or activators of G6PD activity could account for these differences since a mixture of 3460 and HAB enzyme extrapolates intermediate to the two assayed individually (Fig. 1*a*). This enzyme mixture contained a G6PD activity ratio of 2:1 (3460:HAB), expected to yield, therefore, a value of 69% heat labile (using the value of 59% and 91% heat labile G6PD for 3460 and HAB cells, respectively). The extrapolation was to 66% heat labile. As human G6PD is stabilised in the presence of NADP[14], we have examined the ability of NADP to stabilise G6PD against heat denaturation. When 3460 enzyme was incubated with ⩾ 10 μM NADP for 20 min at 4 °C before heat denaturation, we detected no inactivation of G6PD at 50 °C for up to 30 min, whereas ⩾ 50 μM NADP is required to stabilise fully B4 and HAB cell G6PD at this temperature (in the presence of 25 μM NADP, HAB enzyme was 25% denatured within 20 min). In as much as NADP is shown to stabilise all three enzyme preparations to some extent, it was possible that heat lability differences were due to different intracellular amounts of NADP and, thus, differential inactivation during isolation. Therefore, enzyme preparations were isolated in the presence of 1 mM NADP and heat inactivation studies carried out at 60 °C. These results are shown in Fig 1*b*, from which we calculate the fraction

Table 1 Properties of G6PD from BUdR-substituted cells

Enzyme (cell line)	% Heat labile*	K_m† NADP (μM)	G6P (μM)	dG6P utilisation‡
3460	59,56,58,53 (56.5)	14.5±1	33±2	7.5±0.2
B4	77,66,69,67 (69.7)	20.7±1	33±2	8.9±0.2
HAB	91,74,78,76 (79 7)	23 0±1	33±2	10.7±0.3

*Values are from four different enzyme preparations and their mean (shown in parentheses).

†K_ms were determined after dialysis of the enzyme preparations (isolated in the absence of NADP) against 250 mM sucrose, 1 mM EDTA and 1 mM 2-mercaptoethanol for 16 h at 4 °C. NADP was assayed from 1 to 200 μM and G6P from 5 to 300 μM, in triplicate (± s.d.)

‡Percentage of glucose-6-phosphate utilisation (determined in triplicate from two enzyme preparations, ± s.d.), after dialysis for 16 h.

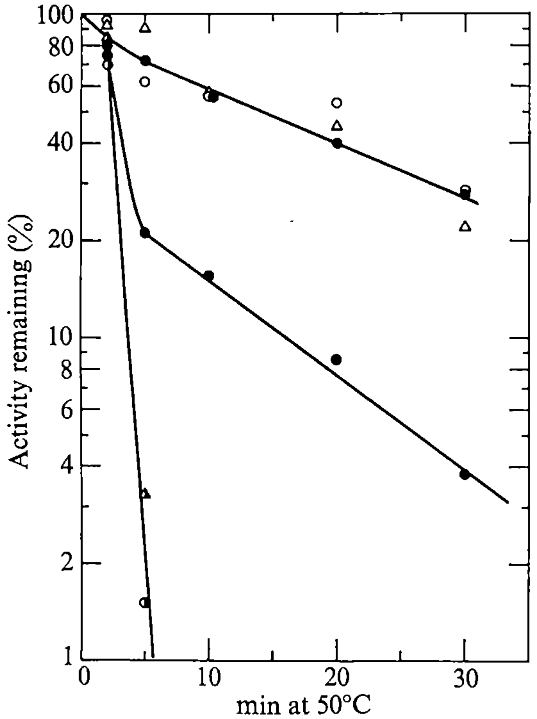

Fig. 3 Heat inactivation of G6PD activities I and II. Enzyme samples (isolated in the absence of NADP) were heated at 50 °C for various times as described in Fig. 1*a* and then subjected to electrophoresis as described in Fig. 2. Gels were stained for G6PD activity for 45 min at 37 °C, fixed in 7% acetic acid, and then scanned at 540 nm to measure the rate of loss of activities I and II as a function of heating time. All values are plotted as the % activity in the respective peaks relative to unheated controls (the average of quadruplicate gels). ●, 3460; ○, B4; △, HAB. Top curve, peak I; lower two curves, peak II.

of heat-labile G6PD in B4 and HAB cells to be 62% and 91%, respectively. In these conditions, 3460 enzyme decayed linearly.

Criteria used to characterise the large number of human G6PD variants[17] include altered *p*H optima, altered K_m for NADP and glucose-6-phosphate (G6P), and the utilisation of 2-deoxy-D-glucose 6-phosphate (dG6P), an analogue of G6P Table 1 shows that all three cell lines had the same K_m for G6P, but differed in their K_m for NADP. Furthermore, B4 and HAB cell G6PD had a slightly greater ability to utilise dG6P as a substrate. All three enzymes showed *p*H optima at 7.5 and 8.5 with only minor differences at the extremes of the curves (*p*H 7.0 and 10.0), not unlike many of the human variants[17].

We could not detect different electrophoretic mobilities of G6PD from the three cell lines on cellulose acetate (frequently used to detect human variants) and have used instead polyacrylamide disk gels, where we have been better able to quantitate enzyme activity on the gel (Fig. 2). Two major peaks of activity were observed for all three cell lines with R_f of 0.22 (peak 1) and 0.28 (peak 2), although the relative proportions of peaks 1 and 2 differed significantly. Thus, the relative amounts of peaks 1 and 2 were routinely found to be 69:31, 52:48, and 28:72 for 3460, B4 and HAB cell G6PD, respectively. (These values varied by ±5% on duplicate gels and with different preparations.)

The increasing proportion of peak 2 in B4 and HAB cell G6PD suggests the possibility that this activity is largely responsible for the increasing fraction of heat labile G6PD in BUdR substituted cells. This possibility was tested by heating the enzyme samples at 50 °C for various times, electrophoresing the enzyme, and determining the fraction of activity remaining in each of the two activity peaks (Fig. 3). Peak 1 decayed at approximately the same rate for all three cell lines. Peak 2 from 3460 cells decayed at a somewhat greater rate than peak 1, whereas peak 2 from B4 and HAB cells decayed substantially faster. Only negligible activity remained after 5 min at 50 °C, making quantitation of activity on the gels difficult.

We have sought to confirm the presence of an altered population of G6PD molecues in B4 and HAB cells using the histochemical staining method described by Wajntal and DeMars[18]. In particular, we have used this assay to determine if a higher proportion of B4 and HAB cells in monolayer can be shown to utilise the substrate analogue dG6P. After growth in monolayer for 24 h, all three cell lines were stained for G6PD activity using G6P or dG6P, or in the absence of substrate. Cells were scored as being stained or unstained at both low magnification and high magnification. This analysis provides an absolute number for cells staining positive under high magnification (where cells with only a few blue granules of formazan are readily discernable), as well as information on the relative intensity of staining (as the ratio of cells staining positive at high magnification/low magnification).

The percentage of cells stained positive for G6PD activity in the presence of both substrates is given in Table 2. Under low magnification, an equivalent number of cells stained positive (~76%) in the presence of G6PD for all three lines, whereas about twice as many B4 and HAB cells were positive in the presence of dG6P compared with 3460. Fewer than 1.0% of the cells were positive in the absence of substrate. Since the scoring of cells at low magnification is somewhat subjective, a better analysis of positive cells was obtained under high magnification. In these conditions the percentage of positive cells in the absence of substrate increased, and there were significant differences between the substituted and unsubstituted cell lines, with 14% of the HAB cells scored positive compared with 5.9% of 3460 cells. This may reflect higher endogenous levels of G6P in the slower growing B4 and HAB cells. Nevertheless, the number of positive cells in the presence of either G6P or dG6P increased under high magnification and when corrected for the difference in background staining (minus substrate), all three cell lines had an equivalent number of cells stained positive in the presence of G6P. After correction for background staining, however, B4 and HAB cells had a substantially higher propor-

Table 2 Percentage of cells positive for G6PD activity

	Cell line		
Substrate	3460	B4	HAB
	Low magnification (×125)		
G6P	73.3 (1,943)	76.6 (1,919)	78.5 (1,867)
dG6P	11.1 (1,473)	21.1 (1,325)	22.5 (1,146)
None	<1.0 (937)	<1.0 (889)	<1.0 (1,042)
	High magnification (×500)		
G6P	86.3 (1,212)	92 4 (956)	93.9 (872)
dG6P	26.7 (1,077)	62.4 (996)	69.0 (919)
None	5.9 (702)	12.9 (726)	14.0 (714)
	Corrected*		
G6P	80.3	79.5	79.9
dG6P	20.7	49.5	55.0
	High magnification/low magnification ratio		
G6P	1.09	1.04	1.02
dG6P	1.86	2.35	2.44

Cells in monolayer were stained for G6PD activity essentially as described by Wajntal and DeMars[18]. Flaskettes (Lab-Tek) were innoculated with 10^5 cells which were grown for 24 h. Media was then removed and each Flaskette was rinsed with 5 ml of 0.9% NaCl. Staining solution (2.5 ml) was added and the Flaskettes were incubated for 1.5 h at 37 °C. The staining solution consisted of: 0.1 M Tris *p*H 7.8, 0.5 mM $MgCl_2$, 0.2 mM NADP, nitroblue tetrazolium (0.5 mg ml $^{-1}$), phenazine methosulphate (0.02 mg ml^{-1}) and 6 mM G6P or dG6P (or no substrate). The plastic cover was removed from the Flaskette and the slide was rinsed in distilled water and counterstained in alcoholiceosin. Cells were scored for blue deposits of formazan at either ×125 or ×500. Numbers of cells scored are given in parentheses.

*Percentage of cells scored positive in the absence of substrate at high magnification subtracted.

tion of cells (× ~2.5) stained positive with dG6P as substrate when compared with 3460 cells. Using the ratio of positive cells at high magnification/low magnification, all three cell lines stained similarly in the presence of G6P (ratio ~1.1), whereas in the presence of dG6P this ratio increased in the B4 and HAB cells, suggesting an increasing fraction of lightly stained cells in the BUdR substituted populations. This consistency in the ratio of high magnification/low magnification in the presence of G6P for all three cell lines suggests that this difference was not due to higher endogenous G6PD levels in the B4 and HAB cells.

Thus, B4 and HAB cells contain a greater fraction of heat labile G6PD molecules than the parent, unsubstituted, cell line. When enzymes are isolated in the absence of NADP, the 3460 cells also have a high level of heat labile G6PD. Using 56.5% heat labile G6PD in 3460 cells (Table 1) as a baseline and 79.7% heat labile G6PD in HAB cells as the effect of 100% BUdR substitution, we would predict that if BUdR is randomly generating mutations or transcriptional errors (which would be a function of the level of BUdR substitution), cells which are 62% substituted (B4) should contain a population of G6PD molecules of which 69.7% are heat labile. We found an average of 70.8%. When enzymes are isolated in the presence of NADP, 3460 G6PD shows a linear decay whereas B4 and HAB are still 62% and 91% heat labile, respectively. Using the extreme values of 3460 (0%) and HAB (91%), we would predict 56% heat labile for B4 on the basis of BUdR substitution, not far from the 62% observed.

Perhaps the clearest demonstration of an altered G6PD in B4 and HAB cells is the difference in the relative amounts of peaks 1 and 2 obtained by electrophoresis on polyacrylamide gels, and the substantial difference in the heat lability of peak 2. Bakay and Nyhan[19] separated human G6PD into three bands of activity by polyacrylamide disk gel electrophoresis, with mobilities similar to the two major bands resolved from the cells used here. The bands of human G6PD activity are interconvertible by NADPH-producing substances, suggesting that the bands are conformers with and without bound NADP[20]. Furthermore, agents which adversely affect the binding of NADP to the enzyme (such as NADPH) promote formation of the more rapidly migrating band. We therefore propose that peak 2 of G6PD activity in the cells examined here represents the enzyme form without bound NADP. When gels were run in the presence of NADPH, the enzymes from all three cell lines migrated as a single band with an R_f of 0.28, similar to peak 2 (unpublished observations). This finding is consistent with the B4 and HAB cell G6PD being deficient in NADP binding.

The fact that B4 and HAB cell G6PD has a higher K_m for NADP and an increasing proportion of peak 2 activity which is extremely heat labile suggests the presence of an altered ("variant") population of G6PD molecules, at least some of which may have altered in their NADP binding by amino acid substitution. The correlation between the fraction of heat labile G6PD and the extent of BUdR substitution suggests that BUdR may be acting as a mutagen, generating a number of variant G6PD molecules in the B4 and HAB cell lines, or alternatively, that these "variants" represent the products of transcriptional errors induced by BUdR. Although we have been unable to detect transcriptional errors by direct nucleotide analysis[6], the analysis of enzymes as reported here is expected to be a more sensitive method of detecting a low level of base (and hence, amino acid) changes. A mutagenic role for BUdR (including transcriptional errors) is somewhat paradoxical in light of the continued viability of the B4 and HAB cell lines. While it seems to offer the most satisfactory explanation of our results, several other enzymes from these cell lines will have to be examined before this question can be answered conclusively.

We thank Bryan Cullen for technical assistance.

Note added in proof: Recent experiments have ruled out a heritable mutational origin for the faulty G6PD and suggest a transcriptional origin.

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Received October 20, 1975; accepted January 9, 1976.

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Decreased levels of an inhibitor of prostaglandin E 9-ketoreductase activity in chick dystrophic breast muscle

A WIDE range of membrane anomalies is one of the characteristics of dystrophic muscle[1,2]. Since prostaglandins (PGs) are known to interact with such membrane constituents as adenyl and guanyl cyclase; Mg^{2+}-dependent, Na^+/K^+-activated ATPase; Mg^{2+}-dependent, Ca^{2+}-activated ATPase; adenylate kinase; and phosphodiesterase[3], it seemed desirable to search for a possible relationship between PGs and the dystrophic condition. The activities of two potentially regulatory PG-metabolising enzymes were measured. NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase activities[4] in the breast muscles of 10-d post-hatching chicks with hereditary muscular dystrophy (New Hampshire strain) and the breast muscles of 10-d post-hatching normal (white leghorn) chicks were the same as those in the leg muscles of these animals. PGE 9-ketoreductase activity[5,6] in the dystrophic breast muscle was considerably more active than its control (normal) breast muscle. Subsequently, it was found that the normal breast muscle contains a much higher level of an inhibitor of PGE 9-ketoreductase activity than dystrophic breast muscle.

The assay system used to measure PGE 9-ketoreductase activities consisted of increments of cytoplasmic fractions of the muscle extracts, 1.0 μg PGE_2, and 1.0 mM NADPH. Enzymatic generation of $PGF_{2\alpha}$ was measured by radioimmunoassay with antibodies directed against $PGF_{2\alpha}$. When the cytoplasmic fractions were tested for their effect on the activity of exogenous PG 9-ketoreductase, the enzyme purified from chicken heart[5] was used. This amount of PGE_2 9-ketoreductase catalysed the generation of 50 ng $PGF_{2\alpha}$ after incubation for 15 min at 37 °C. In these experimental conditions, the initial velocity of the catalytic reaction of the purified enzyme is being measured. Analyses of these reaction mixtures by thin-layer chromatography confirmed the enzymatic conversion of PGE_2 to $PGF_{2\alpha}$. Extracts of the various breast and leg muscles from normal and hereditary dystrophic chicks were made as follows: chicks were decapitated, and the muscles excised and immediately frozen in liquid nitrogen. For extraction, the individual muscles were suspended in 0.092 M phosphate buffer, *p*H 7.3, 0.1 mM dithiothreitol (1.0 g muscle per 4.0 ml buffer) and homogenised using a Sorval Omni homogeniser at full speed for 1 min at

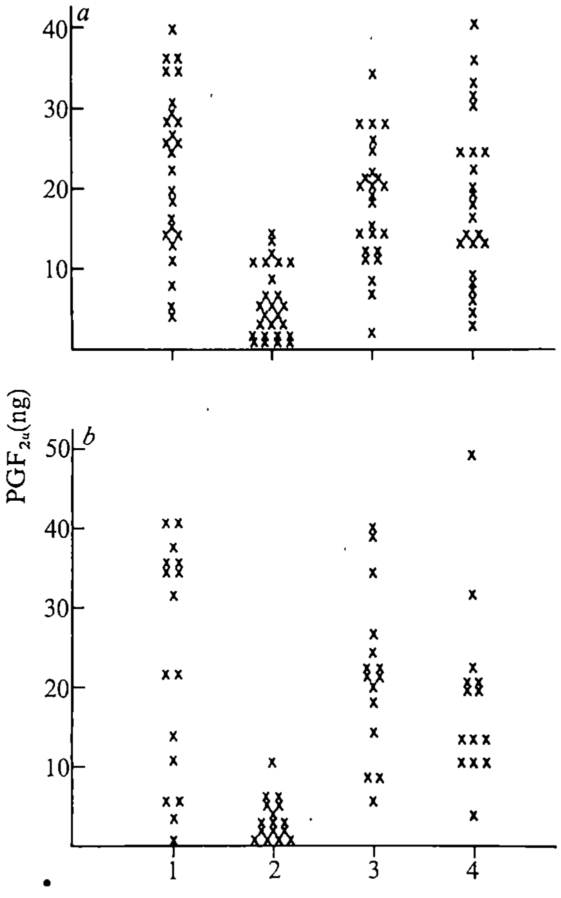
0 °C. After stopping the mixer for 5 min, the muscle suspension was homogenised again for an additional minute. After centrifugation at 10,000g for 20 min, the supernatant fluid was recentrifuged at 100,000g for 1 h, and this supernatant fluid used as the cytoplasmic fraction. Analyses of these cytoplasmic fractions for protein showed that the efficiencies of protein extraction from the four muscles were similar.

PGE 9-ketoreductase activities in terms of ng $PGF_{2\alpha}$ generated from 1.0 μg PGE_2 by 50 and 100 μl cytoplasmic fractions from normal and dystrophic breast muscle (10-d post-hatching), and from the leg muscles of these same chicks, are shown in Fig. 1. Values for the dystrophic breast muscle fall into the same range as those for normal leg muscles and symptom-free leg muscles from the dystrophic chicks. Values for normal breast muscles, on the other hand, are clustered around a much lower level. On increasing the amount of muscle extract from 50 to 100 μl (Figs 1*a* and *b*, and 2*a*) added to the reaction mixture, a disproportionately small increase (even a decrease with normal breast muscle) in $PGF_{2\alpha}$ formation was observed, indicating the presence of an inhibitor in these muscle extracts.

To test this possibility, the effect of extracts from the four types of muscle from two chicks on PG 9-ketoreductase purified from chicken heart was studied. The extracts from normal breast muscle (both the 10-d post-hatching chick and an adult normal chicken) depressed the activity of this preparation considerably more than extracts from dystrophic breast muscle and the leg muscles (Fig. 2*b*). Addition of increasing amounts of extract from the normal breast muscles of the young chicks to the purified PGE 9-ketoreductase first increased enzymatic activity, presumably due to PGE 9-ketoreductase present in the extract, then actually decreased the reductase activity. In the presence of extracts of dystrophic muscles and the leg muscles, reductase activity was also increased; but with higher concentrations of these extracts, the degree of increase in reductase activity was reduced, and in the presence of 100 μl the reductase activity was even inhibited. If the inhibitor in the dystrophic breast muscle and the leg muscles is identical to that present in the normal breast muscle, the normal breast muscle contains 5 to possibly 50 times more of it. An inhibitor of PGE 9-ketoreductase has been partially purified from normal adult breast muscles of chickens. This inhibitor seems to contain a flavin-related moiety. Whether it is identical to the inhibitor found at reduced levels in dystrophic breast muscle is still to be demonstrated.

The effect of 50 μl of the four muscle extracts from several chicks on purified PGE 9-ketoreductase activity is shown in Fig. 3. Again it can be seen that levels of inhibition of the reductase by normal breast muscle are much higher than that of dystrophic breast muscles or the leg muscles. The findings

Fig. 1 Enzymatic production of $PGF_{2\alpha}$ by 50 (*a*) and 100 μl (*b*) cytoplasmic fractions of four muscle preparations from individual animals. 1 and 2, Breast muscle: 1, dystrophic chick; 2, normal chick. 3 and 4, Leg muscle: 3, dystrophic chick; 4, normal chick. Enzymatic reaction mixtures, containing the cytoplasmic fractions, 1.0 μg PGE_2 and 1.0 mM NADPH in a total volume of 0.1 ml, were incubated for 15 min at 37 °C. The reaction was stopped by boiling for 2 min. After addition of 0.9 ml buffer, the denatured protein was removed by centrifugation, and $PGF_{2\alpha}$ estimated by radioimmunoassay[4]



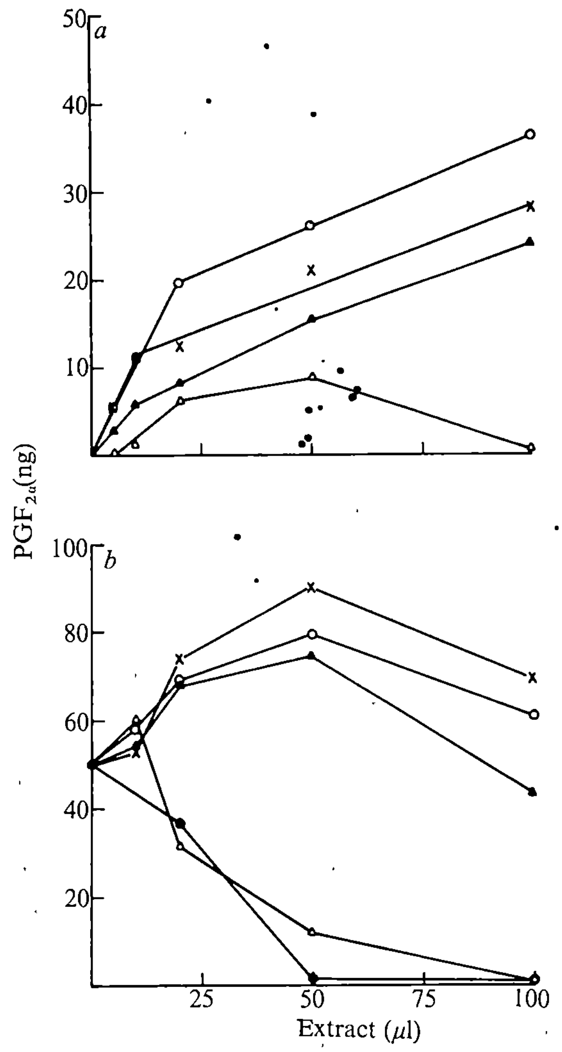


Fig. 2 Enzymatic production of $PGF_{2\alpha}$ by increments of cytoplasmic fractions from four muscle preparations from two chicks (*a*) and these same cytoplasmic fractions in the presence of a constant amount of PGE 9-ketoreductase purified from chicken heart (*b*). ×, Dystrophic breast muscle; △, normal breast muscle; ▲, normal leg muscle; ○, symptom-free leg muscle from dystrophic chick; ●, normal breast muscle from adult chicken. Experimental conditions in *a* are the same as those described in Fig. 1. In *b*, increments of cytoplasmic fractions, purified PGE 9-ketoreductase, 1.0 μg PGE_2 and 1.0 mM NADPH in a volume of 0.1 ml, were incubated for 15 min at 37 °C. After boiling for 2 min followed by addition of 0.9 ml buffer and centrifugation, the supernatant fluid was assayed for $PGF_{2\alpha}$ by radioimmunoassay.

that the low activities of NAD+-dependent 15-hydroxyprostaglandin dehydrogenase in our experimental conditions are the same in normal and dystrophic breast muscle extracts suggest that the increased $PGF_{2\alpha}$ levels in the presence of the dystrophic muscle extracts did not reflect differences in enzymatic oxidation of the 15-hydroxy group.

The presence of different levels of inhibitor of PGE 9-ketoreductase can be added to the properties which distinguish the normal pectoralis (white) muscle from the thigh and leg (red) muscle of the chick[7]. In contrast, this distinction is absent in the dystrophic pectoralis which shows a low level of the inhibitor similar to that of the leg muscle. The properties which distinguish pectoralis (white) leg or heart (red) muscle appear during the last third of embryonic development and the first month of post-hatching. The retardation of the full development of the characteristics of white muscle in the dystrophic pectoralis has been demonstrated with regard to several cell properties[8]. Similarly, one could see in the absence of the PGE 9-ketoreductase inhibitor in dystrophic pectoralis another instance of a suppression of late development.

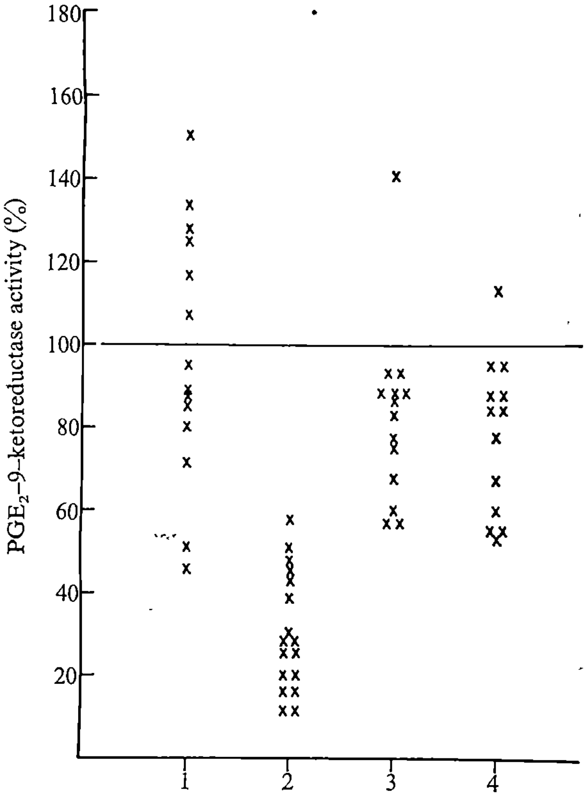


Fig. 3 PGE 9-ketoreductase activity of 50-µl cytoplasmic fractions of the four muscle (for code, see Fig 1) extracts from individual chicks in the presence of a constant amount of PGE 9-ketoreductase purified from chicken heart Enzyme activity is expressed as percentage of that obtained with purified enzyme. Experimental conditions are the same as those in Fig 2*b*.

Different profiles of prostaglandin metabolism have been found in the lung and kidney of foetal and early newborn rats and in adult rats[9,10]. It is possible that the PG system, which includes generation of endoperoxides, thromboxanes, and the primary PGs, have an important role in differentiation. The fact that certain nutritional muscle dystrophies can be induced by a deficiency of an antioxidant such as tocopherol[11], that in these conditions peroxidation of lipids can be demonstrated[12], and that progress of chick dystrophy is retarded by sulphydril-protecting substances such as penicillamine[13], could point to involvement of PG-derived peroxides in the dystrophic degeneration of chick breast muscle. How the relative levels of PGE and $PGF_{2\alpha}$ or the altered PGE 9-ketoreductase activity in the dystrophic breast muscle modulates the thromboxane pathway or endoperoxide stability (if they do indeed have a regulatory role) is not clear.

We thank the US Public Health Service (National Institute of Child Health and Development) and the Muscular Dystrophy Association for support; and Dr L. J. Pierro (Department of Animal Genetics, University of Connecticut) for making the dystrophic and control chickens available to us. L.L. and H.H. are American Cancer Research Society Professors.

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Received January 20, accepted March 5, 1976

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C fragment of lipotropin has a high affinity for brain opiate receptors

THE C-terminal region of lipotropin, named C fragment[1], has been found as an intact polypeptide in the pituitary gland[2] and the C fragment is also released from lipotropin by mild digestion with trypsin *in vitro*[1]. These data suggest that the C fragment of lipotropin might be elaborated *in vivo* in order to perform a physiological function.

Recent reports have shown that in pituitary there is an endogenous peptide with opiate activity[3,4] and similar peptides have been found in brain[5–7]. One of the brain peptides, termed methionine enkephalin, was isolated and described as a pentapeptide with the sequence Tyr–Gly–Gly–Phe–Met[8], which is the same as the sequence at the NH_2-terminus of the C fragment of lipotropin. Although lipotropin[9] and its C fragment[1,2] were isolated from pituitary whereas the pentapeptide was isolated from brain[8], it nevertheless seems probable that methionine enkephalin is derived *in vivo* from lipotropin C fragment. We have therefore determined whether C fragment, and a series of peptides that might be formed from C fragment *in vivo*, has a high affinity for brain opiate-binding sites. The results show that several of the peptides bind strongly to brain opiate receptors and, in particular, that C fragment has a higher affinity than the pentapeptide methionine enkephalin.

Lipotropin, C fragment (residues 61–91 of lipotropin), C′ fragment (61–87), β-melanotropin (41–58) and N fragment (1–38) were isolated from pig pituitary as described previously[1]. The peptide representing residues 61–89 was isolated from a carboxypeptidase A digest (Worthington) of C fragment. The octapeptide and nonapeptide representing residues 61–68 and 61–69 of lipotropin were obtained by enzymic digestion of C fragment with *Armillaria mellea* protease (Arven) and TPCK-trypsin (Worthington) respectively; the peptides were isolated by ion-exchange chromatography on columns of Dowex 50-X2 with volatile pyridine buffers and were desalted on Sephadex G-10. The fragment containing residues 70–79 of lipotropin was isolated from the tryptic digest of C fragment. The peptides were characterised by N- and C-terminal analysis[10,11] and by amino acid composition[12]. The tetrapeptide and pentapeptide representing residues 61–64 and 61–65 of lipotropin were prepared by solid phase peptide synthesis[13]; the products were purified by chromatography on Sephadex C-25 and Sephadex A-25 and chemical and optical purity were confirmed by thin-layer chromatography and by digestion with aminopeptidase M (Röhm).

The peptides were tested for their ability to reduce the binding of the radiolabelled opiate agonist, ^{3}H-dihydromorphine, and the antagonist ^{3}H-naloxone to a membrane preparation from rat brain. The experimental details are given in Table 1. The concentrations of peptides that reduce the specific binding by 50% (I_{50}) are shown, together with the corresponding values for four opiate drugs. The determinations were made at 100 mM sodium concentration, conditions in which the I_{50} values for displacement of bound naloxone have been shown to correlate with pharmacological potency[14].

C fragment at low concentration was effective in displacing specifically bound ^{3}H-naloxone. The I_{50} value (2.6×10^{-9} M) was one tenth of that of morphine or dihydromorphine and was comparable with that of naloxone. Methionine enkephalin (61–65) and the intermediate peptides, 61–68, 61–69 and 61–87, were approximately 30 times less potent than C fragment. The I_{50} values of the closely related peptides 61–87 and 61–89 differed by a factor of 20, indicating the importance of the paired lysine residues (88 and 89) to binding affinity. Lipotropin, which includes the C fragment sequence, and seems to be the precursor of C fragment[15], did not inhibit the binding of ^{3}H-naloxone or ^{3}H-dihydromorphine, and fragments of lipotropin that lacked the C-terminal sequence were also inactive.

When the I_{50} values were obtained from competition

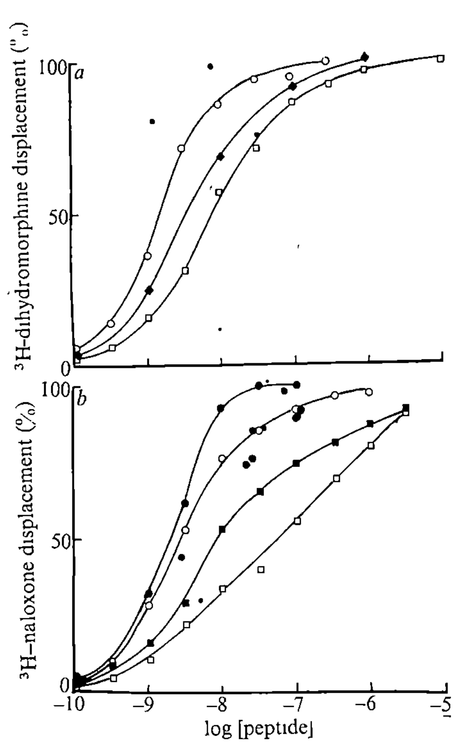

Fig. 1 *a*, Displacement of specifically bound ^{3}H-dihydromorphine by C fragment (○), methionine enkephalin (□) and morphine (◆) in Tris-HCl, (*p*H 7.4), 100 mM NaCl. Incubation conditions are described in the legend to Table 1. *b*, Displacement of specifically bound ^{3}H-naloxone by C fragment (circles) and methionine enkephalin (squares) Open symbols refer to incubations in Tris-HCl, (*p*H 7.4), 100 mM NaCl; closed symbols refer to incubations in Tris-HCl (*p*H 7.4), 100 mM KCl. KCl was included in the 'low sodium' incubation to distinguish the specific effects of sodium from nonspecific ionic strength effects. The effect of substitution of sodium by potassium in the incubation medium is to increase the specific ^{3}H-dihydromorphine counts bound by 30–40% and to decrease the specific binding of ^{3}H-naloxone by 50% Note that, in general, these curves do not represent simple mass action binding processes.

Table 1 The binding of lipotropin fragments to brain opiate receptors

Residue Nos.	Name	I_{50} against ^{3}H-naloxone M × 10^{9}	I_{50} against ^{3}H-dihydromorphine M × 10^{9}	Potency ratio: I_{50} naloxone / I_{50} dihydromorphine
61–91	C fragment	2.6	2.2	1.2
61–89		5	2.2	2.3
61–87	C′ fragment	100	42	2.5
61–69		60	21	2.9
61–68		160	45	3.6
61–65	Methionine enkephalin	90	8	11
61–64		1,000	300	3.3
1–91	β-LPH	⩾ 1,000	⩾ 1,000	—
1–58	γ-LPH	⩾ 1,000	⩾ 1,000	—
1–38	N-Fragment	⩾ 1,000	⩾ 1,000	—
41–58	β-MSH	⩾ 1,000	⩾ 1,000	—
70–79		⩾ 1,000	⩾ 1,000	—
	Morphine	25	4.1	6
	Levorphanol	10	2.0	5
	Dihydromorphine	22	3.6	6
	Naloxone	1.8	2.4	0.7

Opiate binding assays were carried out on a hypotonically lysed (10 mM Tris-HCl, *p*H 7.4), extensively washed, crude synaptosome preparation from whole supratentorial rat brain. The membranes (0.5 mg protein ml^{-1}) were preincubated for 15 min at 30 °C in a buffer containing 50 mM Tris-HCl (*p*H 7.4) and either 100 mM NaCl or KCl. Tritiated opiates (G)-^{3}H-naloxone (New England Nuclear, 20 Ci mmol^{-1}, 1.0×10^{-9}M) or 1,7,8-^{3}H-dihydromorphine (Radiochemical Centre, Amersham, 90 Ci mmol^{-1}, 3.5×10^{-10} M) and appropriate concentrations of unlabelled opiates or peptides were added to 1-ml aliquots of preincubated membrane suspension in plastic microcentrifuge tubes After 15 min incubation in the dark, the suspension was centrifuged (14,000*g*, 2 min), the pellets rapidly and superficially washed with 100 mM NaCl or KCl, and bound radioactivity assayed by liquid scintillation counting at 30% efficiency. Specific binding was defined as that fraction of the bound radioactivity displaced by morphine (10^{-5} M) or levorphanol (10^{-6} M). Incubations were carried out in quadruplicate and the standard error of the mean of a determination rarely exceeded 3% Of 70,000 d.p.m. ^{3}H-dihydromorphine in each assay, 2,700 d.p.m. are bound specifically and 1,500 d.p.m bound nonspecifically. For ^{3}H-naloxone, of the 46,000 d.p m. in each incubation, 4,500 d.p.m. are bound specifically and 3,000 d.p.m. nonspecifically. The I_{50} value represents the concentration of the compound at which 50% of specifically bound radioactivity is displaced.

experiments against ^{3}H-dihydromorphine (Table 1), C fragment was found to have twice the potency of morphine, and methionine enkephalin had approximately one half the potency (Fig. 1*a*). The tetrapeptide (61–64) and peptides intermediate between C fragment and methionine enkephalin were less potent than the pentapeptide. The active peptides and the opiate alkaloid agonists exhibited higher potencies when assayed against ^{3}H-dihydromorphine than against ^{3}H-naloxone. The change in I_{50}, reflected in the potency ratio (Table 1), was most marked in the case of methionine enkephalin and least for C fragment. Thus C fragment behaves more like the antagonist naloxone than the potent agonists morphine and levorphanol.

Replacement of sodium ions in the assay medium by potassium resulted in a large decrease (ninefold) in the I_{50} value for displacement of bound ^{3}H-naloxone by methionine enkephalin but had only a small effect (1.3-fold) on the I_{50} value of C fragment (Fig. 1*b*). With morphine-like alkaloids it has been observed that a large sodium effect correlates with agonist properties[16–19]. Although such a correlation has not been demonstrated for opiate peptides, the results for methionine enkephalin agree with its reported agonist properties[8]. In contrast, C fragment exhibited a much smaller sodium effect. Experiments to test the pharmacological properties of C fragment are in progress[21].

Preliminary studies of the time course of C fragment binding suggest that the peptide interacts with the receptor more slowly than dihydromorphine. Thereafter, the extent of inhibition of ^{3}H-dihydromorphine binding by C fragment remains approximately constant for 2 h. Inhibition by methionine enkephalin, on the other hand, decreases slowly after 20 min, consistent with slow proteolytic degradation of the pentapeptide. The possibility that C fragment might be weakly active but undergoes proteolytic cleavage to yield a more active peptide is unlikely because the 61–64, 61–65, 61–68, 61–69 and 61–87 peptides were less active than C fragment in the binding assay. Furthermore, degradation by a trypsin-like enzyme is highly unlikely because lipotropin, which readily undergoes cleavage *in vitro* to give C fragment[1], was inactive. Certainly, in our assay conditions, C fragment does not undergo conversion to methionine enkephalin as the binding properties of the two peptides are quite different.

The binding studies indicate that a series of peptides derived from lipotropin can inhibit the binding of naloxone and dihydromorphine to brain opiate receptors. The 'active' peptides all contain the N-terminal sequence Tyr–Gly–Gly–Phe, retaining a potential for the β-bend predicted for the receptor conformation of enkephalin[20]. The most active peptide is C fragment, with an affinity substantially greater than that of methionine enkephalin. The high affinity exhibited by C fragment for brain opiate receptors *in vitro* suggests that this peptide, if present in higher regions of brain, could function *in vivo* as a neurotransmitter or neuromodulator.

We thank Sir Arnold Burgen for advice and assistance, and Pramila Mehta and Derek Massey for technical help. We also acknowledge gifts of morphine from Professor W. S. Feldberg, levorphanol tartrate from Roche Products Limited, Welwyn Garden City, Herts., dihydromorphine from Professor H. W. Kosterlitz and *Armillaria mellea* protease from Dr H. Gregory.

Since this manuscript was submitted, the C fragment has been tested *in vivo* by Professor W. S. Feldberg using direct injection into the 3rd ventricle of the cat: it was found to have potent analgesic properties, the effects lasting several hours[21]. In molar terms, C fragment was approximately 200 times more potent than morphine.

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Received February 21; accepted March 29, 1976.

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Structural and conformational relationships between the enkephalins and the opiates

Two pentapeptides, the enkephalins (Fig. 1*a*), have been isolated from the mammalian brain and have been shown to be potent opiate agonists[1,2]. Further work has demonstrated that the opiate analgesics themselves interact competitively with the receptor sites for these endogenous pentapeptides. This explains in a simple and elegant manner the occurrence of opiate receptors in nervous tissue[3,4]; clearly it was unlikely that they were there to interact solely with the opiates. As the enkephalins and opiates are competing for the same receptors and give a similar pharmacological response it is likely that they have structural and conformational similarities. Hughes *et al.* have shown that both of these small peptides contain a tyrosine residue at the amino terminal position[2]. We consider here the significance of that finding for the conformational relationship between the enkephalins and opiates.

The discovery that tyrosine is in a terminal position rather than in the internal sequence, where the nitrogen atom would be part of an amide linkage and therefore not basic, is of considerable significance. If we make the reasonable assumption that the primary locus of interaction of the above peptides with the opiate receptor is at the tyrosine terminal, then this would explain why the 'tyramine' portion of the tyrosine residue (Fig. 1*a*) is a common feature of many of the most potent groups of opiate analgesics such as the morphines (Fig. 1*b*), morphinans (Fig. 2*a*) and 6,7-benzomorphans (Fig. 2*b*). As several antagonists are derived from the above agonists similar considerations will also apply to these drugs. The 3-OH morphinans (Fig. 2*a*, levorphanol) and 2′-OH-6,7-benzomorphans (Fig. 2*b*, metazocine) are as potent as or more potent than morphine; therefore it is obvious that these simplifications of the morphine skeleton, which are considerable in the case of the benzomorphans, still contain the active moiety. The development of various elegant *in vitro*[3,4] methods of assessing opiate agonist and antagonist activity has removed some of the problems of structure–activity correlations based simply on *in vivo* measurements where factors other than opiate receptor interaction may distort accurate interpretations. The fact that the tyramine fragment of the enkephalins has a primary amino group rather than a tertiary function as in many opiates is perhaps not surprising. Normorphine, which contains a secondary amino group, is as active as morphine in various *in vivo*[5] and *in vitro*[6] systems, thus showing that the presence of a tertiary amino group is not an absolute requirement for agonist activity. The importance of the phenolic hydroxyl group in opiate agonists and antagonists is well known[7,8], and results from various *in vitro* systems[3] show that the methyl ether of morphine, codeine, has only 0.7% of the potency of morphine in the guinea pig ileum and a mere 0.053% of its activity in the rat brain stereospecific binding assay, whereas as an analgesic in man it has about 8% of the activity of the parent drug. In the 6,7-benzomorphan series it has also been shown that replacement of the 2′ phenolic-OH by $-NO_2-NH_2$, Cl or F groups leads to less active agonists[9].

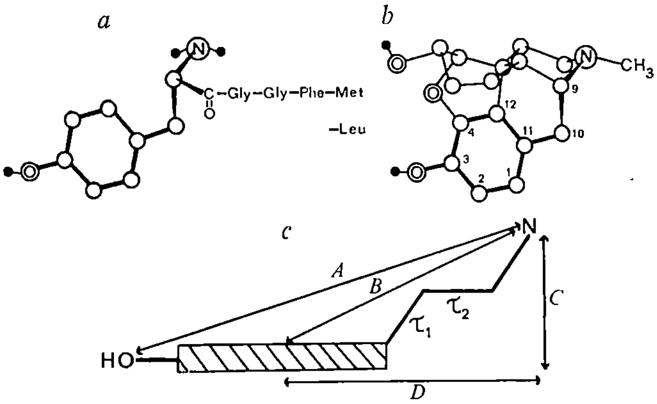

Fig. 1 *a*, Amino acid sequences of the enkephalin mixture[2] with the 'tyramine' moiety of the tyrosine residue in heavy outline. *b*, X-ray structure of (−)-morphine·HI (ref. 17) with the tyramine moiety in heavy outline. *c*, Diagrammatic presentation of a side-on view of the tyramine moiety, the rectangle representing the benzene ring. *A*, Distance of the nitrogen atom from the oxygen atom; *B*, distance of the nitrogen atom from the centre of the aromatic ring; *C*, perpendicular distance of the nitrogen atom above the plane of the benzene ring; *D*, distance of the centre of the aromatic ring from the point of intersection of the plane of the benzene ring and the perpendicular distance *C*. The torsion angle τ_1 is defined as the angle between the planes of the atoms C1–C11–C10 and C11–C10–C9, that is, for rotation about the C11–C10 bond. Torsion angle τ_2 is the angle between the planes of the atoms C11–C10–C9 and C10–C9–N, that is, for rotation about the C10–C9 bond.

Although there is a 2-carbon chain between the benzene ring and the nitrogen atom there are also two possible 3-carbon chains between these two functions in the morphine, morphinan and 6,7-benzomorphan analogues; but preparation of simple 3-carbon amines carrying a *m*-phenolic-OH group produced drugs with only very weak analgesic activity[10]. As well as having a similar molecular fragment, most opiates exhibit stereospecificity of action[7]; essentially all the analgesic activity resides in the (−)-isomer, and the configuration at C9 in (−)-morphine and at the equivalent carbon atoms in the active morphinans and 6,7-benzomorphans is the same[14].

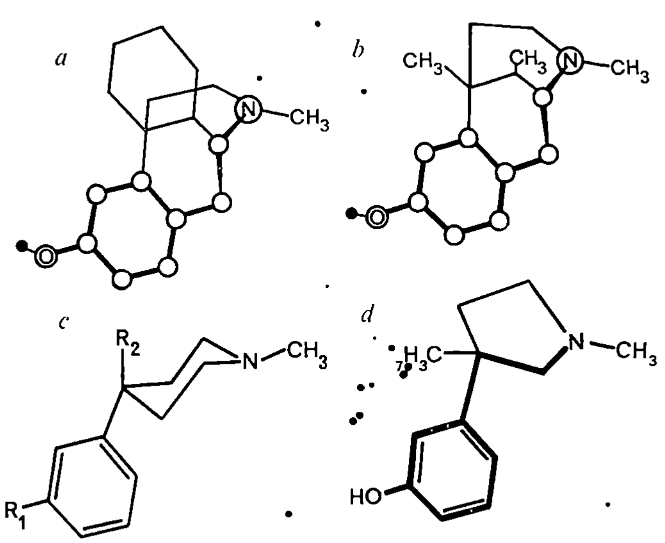

Fig. 2 The opiate agonists levorphanol (*a*) and metazocine (*b*) with the tyramine moiety in heavy outline; *c*, the 4-phenylpiperidines; *d*, profadol with the 2-carbon side chain amine and aromatic ring in heavy outline. ○, carbon atoms; ●, hydrogen atoms.

As the actions of the opiates are stereospecific and they are more or less rigid molecules, one possible way of obtaining information about the most likely opiate receptor conformation of the 'tyramine' portion of the tyrosine residue in the enkephalin pentapeptides is to examine the conformations of the tyramine residue in the potent opiates as determined by X-ray crystallography. The opiates are fairly rigid molecules so the conformation obtained from the X-ray data can confidently be assumed to be very similar to that occurring at the opiate receptor. We have therefore determined certain molecular parameters (Fig. 1*c* and Table 1) for the tyramine fragment in several conformationally restricted opiate agonists and antagonists based on published X-ray data. It is probably reasonable to examine both opiate agonists and antagonists, as there is now excellent evidence that they are combining with the same or very similar receptor sites[3,4,6,11,12]. For the agonists morphine and codeine, the antagonists, naloxone and 2-allyl 5,9-dimethyl-2′-hydroxy 6,7-benzomorphan[13] (*N*-allylnormetazocine) and the agonist–antagonist[6] cyclazocine (*N*-cyclopropylmethylnormetazocine) we find the following mean values for the various molecular parameters $A = 7.0$ Å, $B = 4.5$ Å, $C = 1.2$ Å, $D = 4.4$ Å $\tau_1 = 175°$ $\tau_2 = -89°$. A combination of some of these or closely similar values may correspond to the optimal opiate receptor site conformation for the 'tyramine' fragment of the enkephalins. Further simplifications of the morphine structure has led to molecules having greater degrees of conformational freedom, such as the 4-phenylpiperidines (Fig. 2*c*) and the 3-phenylpyrrolidines (Fig. 2*d*). The former group of drugs is

Table 1 Molecular parameters for various opiate agonists, antagonists and other drugs

	A(Å)	*B*(Å)	*C*(Å)	*D*(Å)	τ_1 (degrees)	τ_2 (degrees)
(−)-Morphine·HI[17]	7.08	4.55	0.81	4.47	164.7	−94.9
(−)-Codeine·HBr[18]	7.06	4.54	0.88	4.45	171.9	−91.4
(−)-Naloxone·HCl[19]	6.96	4.32	1.27	4.12	180.7	−87.3
(±)-2-Allyl 5,9-dimethyl-2-hydroxy 6,7-benzomorphan ·HBr[20] (*N*-allylnormetazocine)	6.89	4.42	1.74	4.06	174.2	−81.3
(−)-Cyclazocine·HBr[21] (*N*-cyclopropylmethyl normetazocine)	7 08	4.55	1.23	4.38	182.6	−94.2
Meperidine HBr[22]		5.71	1.24	5.57		
Tyramine HCl[23]	7.82	5.17	1.36	4.99	109.3	181.3
L-Tyrosine ethyl ester[24]	7.91	5.19	1.32	5.02	108.1	179.9

* The molecular parameters are defined in the legend to Fig. 1. Values *A*, *B*, *C*, τ_1 and τ_2 were all calculated from the published atomic coordinates; the values for *D* were obtained by simple geometry.

based on the piperidine–phenol ring fragment in morphine and it is, therefore, not surprising that the optimal position for the hydroxyl group is *meta* with respect to the piperidine ring[14]. The most active drugs of the 3-phenylpyrrolidine series also have the hydroxyl group in the *meta* position[15]. Although in the tyramine fragment of morphine the hydroxyl group is *para*, models of the 3-phenylpyrrolidines clearly show that the spatial disposition of the nitrogen atom with respect to the benzene ring and hydroxyl group is very similar to that in morphine.

Similar considerations apply to the *m* hydroxyl 4-phenylpiperidines although here models and the *B* distance in meperidine (Fig. 2*c*, $R_1 = H, R_2 = -\overset{\overset{O}{\|}}{C}-OC_2H_5$) indicate that the distances differ from those found for the more rigid opiates. Two of the more widely investigated drugs in this group are the agonists bemidone (Fig. 2*c*, $R_1 = OH$, $R_2 = -\overset{\overset{O}{\|}}{C}-OC_2H_5$) and (−)-profadol (Fig. 2*d*). In *in vitro*[3,6] systems the former has $^1/_{12}$ and the latter $^1/_9$ of the potency of (−)-morphine. It is therefore tempting to ascribe this decrease of potency to the greater degree of conformational mobility and to the fact that the various moieties may not be held in their optimal positions as in the more rigid opiates. This simple concept is complicated, however, by the fact that ketobemidone (Fig. 2*c*, $R_1 = OH$, $R_2 = -\overset{\overset{O}{\|}}{C}-C_2H_5$) both in *in vivo*[7,14] and *in vitro*[3] tests has a similar potency to morphine; the conformation of this drug, in the crystal state and in solution, is worthy of further study. Clearly however, with these less rigid drugs it is unwise to place too much importance on the possible pharmacological significance of values obtained by these methods.

It must be stressed, however, that although the presence of the tyramine fragment may be of importance in partially explaining the action of several groups of agonists and antagonists the stereospecificity/selectivity factor and the contribution of additional modes of binding from other portions of the molecule are obviously also critical[8]. The fact that drugs such as methadone and dextromoramide are quite different from those discussed here, and do not contain a tyramine fragment yet have agonist activities equal to or greater than morphine[7] and are probably acting on the same receptor[3,6,16], reinforces the above suggestions that other structural factors and modes of receptor interaction are obviously also important.

It is well known[7] that the introduction of certain substituents on to the N atom converts the opiates into antagonists, for example, substitution of an allyl group for the methyl group on nitrogen converts the agonists morphine and oxymorphone into the antagonists nalorphine and naloxone, respectively. It would be of interest to see what effect similar substitutions on the nitrogen of the tyramine fragment of the enkephalins produced, that is, would this result in antagonists or just less active molecules? Until there are data on the crystal and solution conformations of the enkephalins and the results of varying the structure are known, it would be premature to speculate on the significance of the rest of the peptide sequence. It seems worthy of emphasis, however, that the 'tyramine' conformation we have suggested might be the preferred one, for these pentapeptides at opiate receptors may not correspond to the preferred conformation for this residue found by X-ray crystallography, NMR spectroscopy or theoretical calculations, because the enkephalin pentapeptides are flexible molecules unlike the opiates and close analogues which are fairly rigid. In this regard, it is of interest that both tyramine HCl and tyrosine ethyl ester in the crystalline state have similar values for some of the molecular parameters (Table 1) but that these differ from those found for the opiates.

After this paper had been accepted for publication an article by Bradbury *et al.* appeared[25] in which they suggested that methionine enkephalin bears no similarity in its primary structure to the opiates; we feel, however, that our theory argues strongly against this.

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Received February 24, accepted March 29, 1976.

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Increased membrane fluidity implicated in acceleration of decay of post-tetanic potentiation by alcohols

AFTER repetitive stimulation of a synapse, the efficacy of synaptic transmission may be potentiated for a period. In systems that lend themselves to detailed analyses, this post-tetanic potentiation (PTP) has been shown to be due to an increased amount of transmitter released by the presynaptic neurone rather than to postsynaptic mechanisms[1]. We have investigated the PTP of a monosynaptic, all-or-none, excitatory postsynaptic potential (e.p.s.p.) recorded in cell R15 of the abdominal ganglion of *Aplysia californica* with appropriate stimulation of the right visceropleural connective, and have presented evidence that the PTP of this e.p.s.p. is based on increased transmitter release[2]. We have shown[3] that while PTP is slightly prolonged by lowering the temperature of the preparation from 20 to 12 °C, the duration of the PTP is increased tenfold by further lowering of the temperature to 10 °C This demonstration of a transition temperature suggested that the fluidity of critical lipids in the presynaptic membrane regulates the decay of PTP, such that PTP decays more slowly in conditions of decreased membrane fluidity. If this inference is correct, agents, such as alcohols[4], that increase membrane fluidity and thus act as 'antifreezes', should either prevent the occurrence of the temperature transition or lower the transition temperature. These agents should also, by themselves, accelerate the rate of PTP decay and their potency should be correlated with their lipophilicity. We present here experimental evidence in support of these predictions.

The abdominal ganglion of *A. californica* was pinned in a dish and perfused with fortified artificial seawater at 15 °C

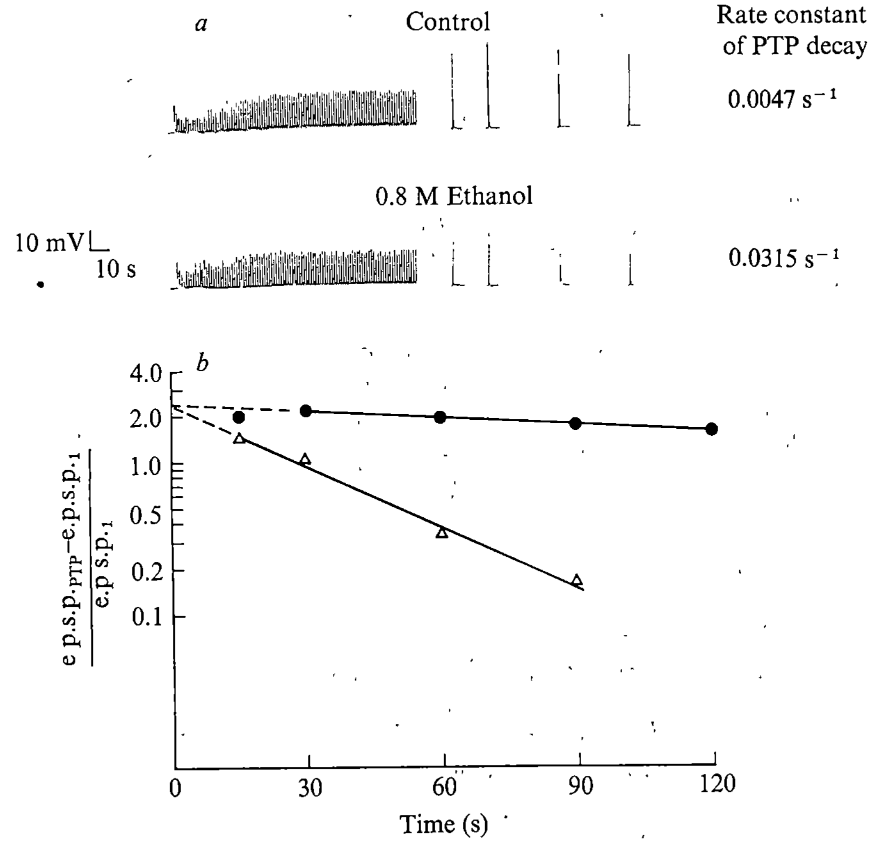

Fig. 1 Bath application of 0.8 M ethanol increases the rate constant of the decay of PTP. *a*, Intracellular record of the e.p.s.p.s recorded in cell R15 of the abdominal ganglion on stimulation of the right visceropleural connective during a train of 100 pulses at 1 per s followed by test pulses at 15-s intervals and then every 30 s. Cell R15 was hyperpolarised to −100 mV by injecting current through the second barrel of a double-barrelled recording electrode. Repetitive stimulation at 1 pulse per s resulted in an initial decrease in the size of the e.p.s.p. (synaptic depression) followed by a gradual increase of the e.p.s.p. amplitude to a facilitated plateau (frequency facilitation). When the train stopped, PTP of the e.p.s.p. amplitude was observed Thirty minutes after a control train ethanol perfusion was begun and a train was given in the presence of ethanol 30 min later. Ethanol had no effect on the amplitude of the e.p.s.p.s during the train but selectively shortened the duration of PTP. *b*, During PTP, the amplitude of the test e p.s.p. (e.p.s.p.$_{PTP}$) returned towards the size of the first e.p.s.p. of a train (e.p.s.p.$_1$) with a single exponential time course. The rate constant of the decay of PTP (the slope of the straight line fitted to the data points after the peak by linear regression analyses) was increased by the application of ethanol. The zero time intercept (a measure of the PTP amplitude) was not affected significantly by ethanol. Data are from a single preparation. ●, Control; △, 0.8 M ethanol.

(ref. 5) to which alcohols were added when indicated. Cell R15 was hyperpolarised to eliminate endogenous bursting; stimulation and intracellular recording were as before[2,5]. The right visceropleural connective was stimulated with trains of 100 pulses at 1 s^{-1}. On termination of the train, two test pulses were given at 15-s intervals to obtain an approximation of the maximal amplitude of the e.p.s.p. during the PTP period. Thereafter test pulses were given at 30-s intervals to obtain an estimate of the duration of PTP.

Perfusion of the preparation with 0.8 or 1.0 M ethanol (in fortified artificial seawater[5]) greatly accelerated the decay of PTP (Fig. 1 and Table 1). The effect on PTP decay was selective, since, on the average, ethanol had no effect on an isolated e.p.s.p., on synaptic depression (the ratio of the amplitudes of the first to the second of a pair of e.p.s.p.s 1 s apart), on frequency facilitation (the ratio of the last to the the first e.p.s.p. of a train) or on the maximum amplitude of the PTP (the zero time intercept of plots like that of Fig. 1*b*) (Table 1). Ethanol has also been seen to increase the rate of decay of PTP at a mammalian neuromuscular junction[1].

We tested the effect of ethanol on the transition temperature below which PTP decays very slowly[3]. In three different experiments, lowering the temperature from 20 °C to 8 °C, in the presence of 0.8 M ethanol, only slightly increased the duration of the PTP, and no evidence was found for the existence of a transition temperature between 12 °C and 10 °C. In each of these three preparations the ethanol was subsequently removed and the duration of the PTP was determined at 12 °C and 10 °C. In the absence of ethanol the PTP was strikingly prolonged on lowering the temperature from 12 °C to 10 °C as had been found before[3]. The fact that ethanol, an agent which promotes membrane fluidity[4], prevents the effect of lowering temperature, supports our conclusion[3] that the transition temperature reflects the sudden decrease in the fluidity of critical presynaptic membrane components.

The inference that ethanol accelerates the rate of decay of PTP by promoting membrane fluidity was further supported by the progressive increase in potency of the series of aliphatic alcohols (ethanol to *n*-octanol) in accelerating the decay of PTP (Fig. 2*a*) The relative potency of the alcohols correlated with their partition coefficient in an *n*-octanol–water system[6] (Fig. 2*b*).

These findings, that alcohols accelerate the decay of PTP, and prevent the previously observed[3] temperature transition in the rate of PTP decay, suggest that the rate is limited by the membrane fluidity of some nerve terminal component. How direct an effect membrane fluidity has on PTP decay rate is unknown. The effect could be fairly indirect. For example, increasing membrane fluidity may activate a membrane-bound enzyme which in turn promotes the decay of PTP. Alternatively, the change in efficiency of transmitter release which directly underlines PTP[2,7,8] may itself be a

Table 1 Effect of ethanol on an isolated e.p.s.p., synaptic depression, frequency facilitation and PTP

	Isolated e.p.s.p. (mV)	Synaptic depression	Frequency facilitation	PTP	Rate constant of decay of PTP (s^{-1})
Control ± s.e.	12.6±2.4	1.11±0.03	1.71±0.23	0.72±0 22	0.0038±0.0006
1.0 M ethanol ± s.e.	13 2±1.9	1.10±0.03	1.40±0.09	0.76±0.26	0.0245±0.0064
P (paired *t* test, $n = 11$)	NS	NS	NS	NS	<0.005

Synaptic depression is defined as the ratio of the amplitudes of the first to the second e.p s.p.s of a train elicited at a frequency of 1 s^{-1}. Frequency facilitation is the ratio of the amplitudes of the last to the first e.p.s.p. of a train of 100 pulses at 1 s^{-1}. To determine the amplitude of PTP and the rate constant of decay of PTP, the first four test e.p.s.p.s given at 30-s intervals starting 15 s after the end of a train were plotted as in Fig. 1*b*. A straight line was fitted to these points by linear regression analyses. The zero time intercept is a measure of the PTP amplitude and the slope of the line represents the rate constant of decay of PTP. NS, not significant.

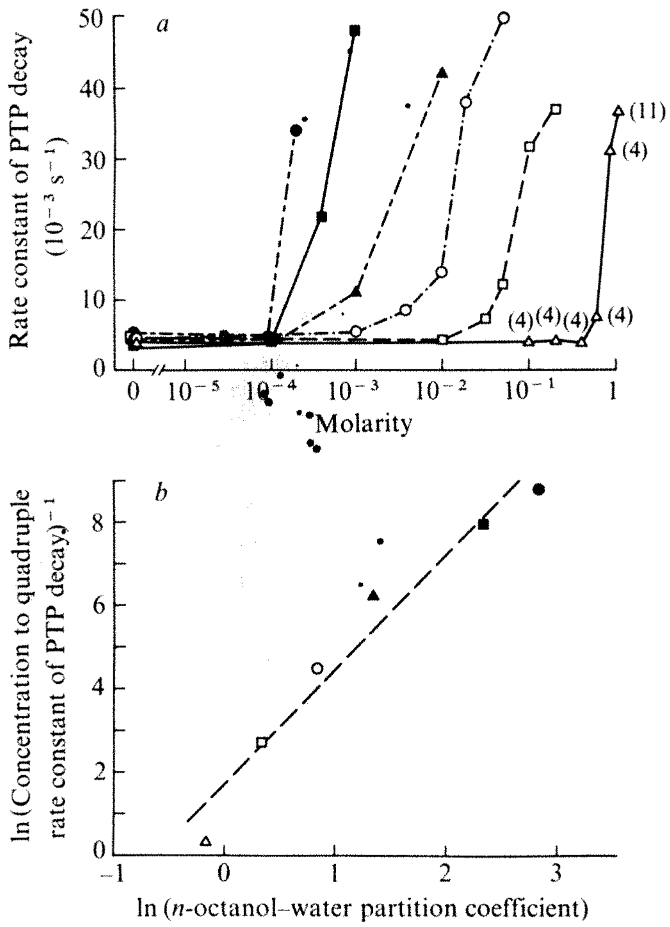

Fig. 2 *a*, Potency of aliphatic alcohols in increasing the rate constant of PTP decay. ●, C_8; ■, C_7; ▲, C_5; ○, C_4; □, C_3; △, C_2. The size of the carbon chain is indicated by the number (C_2 is ethanol; C_8 is *n*-octanol). An aliphatic alcohol (in fortified artificial seawater) was perfused for 30 min, beginning 30 min after a control train; and then a train was given in the presence of the alcohol. In contrast with ethanol, the higher alcohols reduced the amplitudes of all e.p.s.p.s. PTP still decayed, however, with a single exponential time course with a rate constant which was readily determined. Each data point represents the average value obtained from three different preparations, except for ethanol where the number of preparations at each data point is indicated. Note that the effect of each alcohol increases markedly over a narrow range of concentration. *b*, The potency of the effect of the different alcohols (measured as the concentration, *C*, needed to increase the rate of decay of PTP fourfold) correlates with the lipophilicity of the alcohol (as measured by the *n*-octanol : water partition coefficient, *P*). The data points were obtained from (*a*) by interpolating the concentration of each alcohol which would give a rate constant of PTP decay of 20×10^{-3} s^{-1}. A plot of $\ln(1/C)$ against $\ln P$ gives a straight line with the equation $\ln(1/C) = 2.71 \ln P + 1.66$. Other biological systems susceptible to alcohols similarly give straight lines in these coordinates[6]. Symbols as in (*a*).

change in presynaptic membrane organisation (for example, a change in the number or effectiveness of "vesicle attachment sites"[9]). In this latter case, the relaxation of this state of organisation would correspond to the decay of PTP, and would be directly affected by the fluidity of components of the presynaptic membrane. Evidence that this possibility is consistent with other known properties of PTP will be presented later. Regardless of the steps connecting membrane fluidity to the rate of PTP decay, our findings suggest that nerve terminal membrane fluidity is a potential physiological regulation point for the duration of PTP.

We thank Drs S. J. Singer and J. P. Tremblay for their comments and suggestions during the progress of this work, and Mr C. K. Becker for technical assistance. This research was supported by the Veterans Administration Hospital, San Diego, and by a grant from the National Institute on Alcohol Abuse and Alcoholism.

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Single-channel currents recorded from membrane of denervated frog muscle fibres

THE ionic channel associated with the acetylcholine (ACh) receptor at the neuromuscular junction of skeletal muscle fibres is probably the best described channel in biological membranes. Nevertheless, the properties of individual channels are still unknown, as previous studies were concerned with average population properties. Macroscopic conductance fluctuations occurring in the presence of ACh were analysed to provide estimates for single channel conductance and mean open times[1–3]. The values obtained, however, depended on assumptions about the shape of the elementary conductance contribution—for example, that the elementary contribution is a square pulse-like event[2]. Clearly, it would be of great interest to refine techniques of conductance measurement in order to resolve discrete changes in conductance which are expected to occur when single channels open or close. This has not been possible so far because of excessive extraneous background noise. We report on a more sensitive method of conductance measurement, which, in appropriate conditions, reveals discrete changes in conductance that show many of the features that have been postulated for single ionic channels.

The key to the high resolution in the present experiments lies in limiting the membrane area from which current is measured to a small patch, and thereby decreasing background membrane noise. This is achieved by applying closely the tip of a glass pipette, 3–5 μm in diameter, on to the muscle surface, thus isolating electrically a small patch of membrane (Fig. 1). This method has been applied previously in various modifications and mostly with larger pipette tips to muscle[4], molluscan neurones[5,6], and squid axon[7]. The pipette, which has fire-polished edges, is filled with Ringer's solution and contains the cholinergic agonist at micromolar concentrations. Its interior is connected to the input of a virtual-ground circuit, which clamps the potential inside the pipette to ground and at the same time measures current flowing through the pipette, that is, through the patch of membrane covered by the pipette opening. The interior of the muscle fibre is clamped locally to a fixed value by a conventional two-microelectrode clamp[8]. Thus, voltage-clamp conditions are secured across the patch of membrane under investigation. Since current

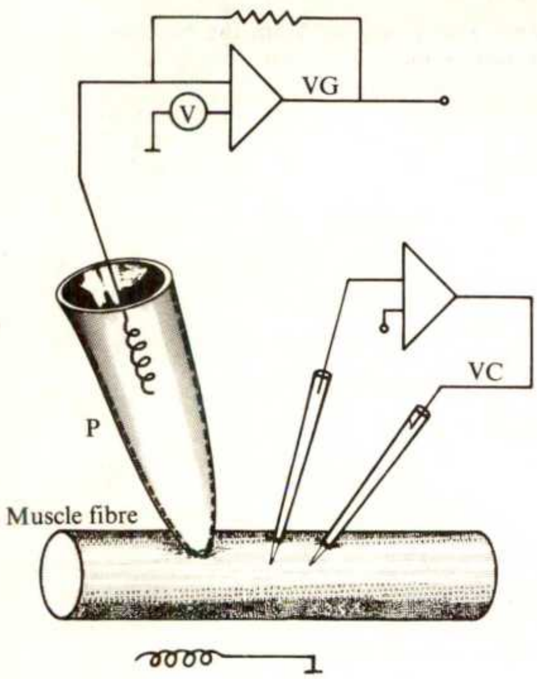

Fig. 1 Schematic circuit diagram for current recording from a patch of membrane with an extracellular pipette. VC, Standard two-microelectrode voltage clamp circuit to set locally the membrane potential of the fibre to a fixed value. P, Pipette, fire polished, with 3–5 μm diameter opening, containing Ringer's solution and agonist at concentrations between 2×10^{-7} and 6×10^{-5} M. d.c. resistance of the pipette: 2–5 MΩ. The pipette tip applied closely on to the muscle fibre within 200 μm of the intracellular clamp electrodes. VG, Virtual ground circuit, using a Function Modules Model 380K operational amplifier and a 500 MΩ feedback resistor to measure membrane current. The amplifier is mounted together with a shielded pipette holder on a motor-driven micromanipulator. V, Bucking potential and test signal for balancing of pipette leakage and measuring pipette resistance.

densities involved are very small, a simple virtual ground inside the pipette is preferable to more complicated arrangements for stabilising potential described previously[6].

The dominant source of background noise in these measurements was the leakage shunt under the pipette rim between membrane and glass. It was constantly monitored by measuring the electrical conductance between pipette interior and bath. Discrete conductance changes could be resolved only when the conductance between pipette interior and bath decreased by a factor of four or more after contact between pipette and membrane. To minimise the leakage conductance, the muscle was treated with collagenase and protease[9]. This enzyme treatment digested connective tissue and the basement membrane, thereby enabling closer contact between glass and membrane. At the same time, however, it made the membrane fragile and more sensitive to damage by the approaching pipette. It did not, however, change the ACh sensitivity of the fibre or alter the properties of ACh-induced conductance fluctuations (E.N. and B.S., unpublished).

All experiments were carried out on the extrasynaptic region of denervated hypersensitive muscle fibres. The uniform ACh sensitivity found over most of the surface of these fibres greatly enhanced the probability of the occurrence of agonist-induced conductance changes at the membrane patch under investigation. Extrasynaptic ACh channels of denervated muscle fibres have mean open times which are about three to five times longer than those of endplate channels[1,10–12]. The longer duration facilitated the detection of conductance changes. Additional measures were taken which are known to either increase the size of the elementary current pulse or prolong its duration: the membrane was hyperpolarised up to −120 mV; suberyldicholine (SubCh) was used as an agonist in most of the experiments; the preparation was cooled to 6–8 °C.

Figure 2 shows a current recording taken in the conditions outlined above. Current can be seen to switch repeatedly between different levels. The discrete changes are interpreted as the result of opening and closing of individual channels. This interpretation is based on the very close similarity to single-channel recordings obtained in artificial membrane systems[13]. The preparation under study is, however, subject to a number of additional sources of artefact. Therefore it is necessary to prove that the recorded events do show the properties which are assigned to ionic channels of the cholinergic system. These are: a correlation with the degree of hypersensitivity of the muscle membrane; an amplitude dependent on membrane potential as predicted by noise analysis; a mean length or channel open time, which should depend on voltage in a characteristic manner[2]; pharmacological specificity with different mean open times for different cholinergic agonists[14,15]. The experiments bore out all of the above-mentioned points as outlined below.

The frequency of occurrence of single blips depended on the sensitivity of the patch under investigation. A plot of the number of current pulses per second against the iontophoretically measured sensitivity of the membrane region, determined either immediately before or after the pipette experiment, revealed a distinct correlation between both quantities with a correlation coefficient of 0.91 for a linear regression (Fig. 3*b*). Student's *t* test assigned a significance better than 0.1% to the relationship.

To estimate the size of the current pulses amplitude histograms were calculated from the current recordings (Fig. 3*c*). The histograms show a prominent peak of gaussian shape around zero deviation, the width of which is a measure of the high frequency background noise of the current trace. Multiple, equally spaced peaks at larger deviations represent the probabilities that either one, two, or three channels are open simultaneously. The peak separation gives the amplitude of the single-channel contribution, which was 3.4 pA for the histogram shown in Fig. 3*c*.

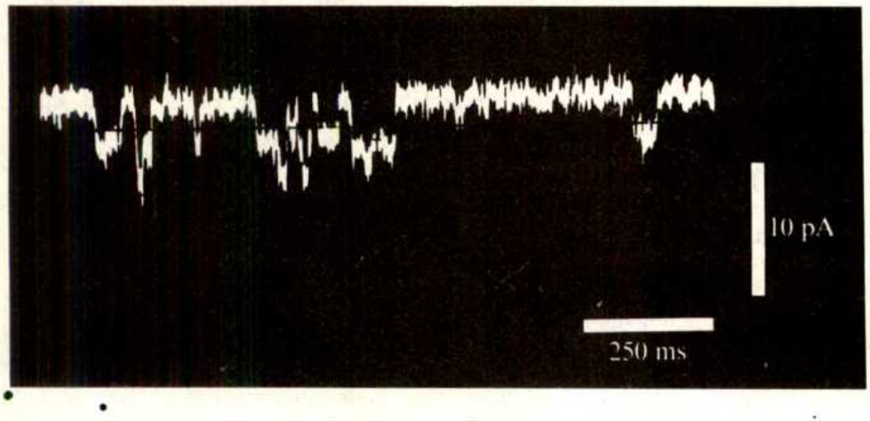

Fig. 2 Oscilloscope recording of current through a patch of membrane of approximately 10 μm². Downward deflection of the trace represents inward current. The pipette contained 2×10^{-7} M SubCh in Ringer's solution. The experiment was carried out with a denervated hypersensitive frog cutaneus pectoris (*Rana pipiens*) muscle in normal frog Ringer's solution. The record was filtered at a bandwidth of 200 Hz. Membrane potential: −120 mV. Temperature: 8 °C.

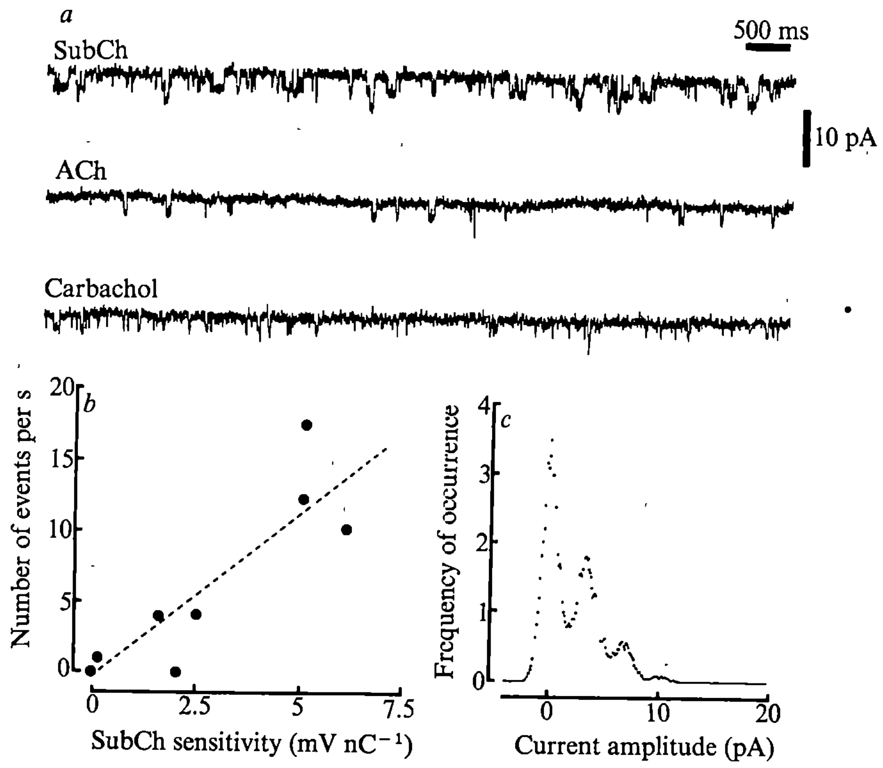

Fig. 3 Characterisation of single-channel currents. *a*, Comparison of current recordings obtained with different cholinergic agonists at concentrations of 2.10^{-7} M(SubCh), 2.10^{-6} M(ACh), and 6.10^{-5} M(carbachol). Downward deflection of the trace represents inward current. Three different experiments. Pen records replayed from analogue tape at a bandwidth of 100 Hz. All experiments at −120 mV membrane potential; 8 °C. *b*, Number of current blips per second is plotted against iontophoretic sensitivity of the membrane region under investigation. Sensitivity was determined by 100-ms iontophoretic pulses delivered from a pipette filled with 1 M SubCh (40 MΩ pipette resistance, 10 nA bucking current, sensitivity measured at resting potential). Pooled data from eight experiments. Broken line represents linear regression. *c*, Amplitude histogram of membrane current. Current traces were digitalised and baselines fitted by eye to data records, each 4 s in length. Frequency of occurrence of deviations from the baseline is shown in arbitrary units. Histograms were calculated on a PDP-11 computer; 2–8 histograms were averaged to obtain curves like the one shown. 8 °C; −120 mV membrane potential.

This was obtained from an experiment at −120 mV membrane potential. A similar histogram from the same muscle fibre obtained at −80 mV yields a current pulse amplitude of 2.2 pA. These two values extrapolate to an equilibrium potential of −7 mV. Channel conductance is estimated as 28 pmhos in this case. It scattered from fibre to fibre with a mean value of 22.4±0.3 pmhos (mean±s.e.; number of determinations=27). This value is somewhat lower than the one derived from noise analysis at normal endplates, which is 28.6 pmhos for SubCh[15]. Higher order peaks in the histograms are not merely scaled images of the zero order peak. They tend to be smeared out due to non-uniformity of current pulse amplitudes. We cannot decide at present whether this is a real feature of the channels or a measurement artefact. Such an effect could arise if not all of the channels are located ideally in the central region of the pipette opening. Current contributions from peripherally located channels would only partially be picked up by the pipette. This source of error is also likely to lead to an underestimate of channel size if the pipette seal is not optimal.

Temporal analysis of the current records was carried out partly by measurement of individual channel length and averaging 40–50 measurements, and partly by calculation of the power spectrum of the current recordings. In the latter case, the cutoff frequency f_0 of the Lorentzian spectrum yielded an estimate of mean channel open time τ (or pulse duration) through the relationship $\tau=1/(2\pi f_0)$. Values of mean open times obtained by the two methods were consistent within ± 30%. For SubCh as an agonist and at a temperature of 8 °C it was 45±3 ms (n=11) at −120 mV and 28±3 ms (n=14) at −80 mV. These values are approximately three times longer than the corresponding mean open times of endplate channels derived from noise analysis[15]. Note, however, that lengthening of channel durations by factors of three to five at extrajunctional sites with respect to endplate values has been measured independently by conventional noise analysis[12]. The voltage dependence of the values given above corresponds to an *e*-fold change per 80 mV, which is within the range of published values[2].

Channel open times were different when different cholinergic agonists were used (Fig. 3*a*). For −120 mV and 8 °C, mean channel open time was 45±3 ms (n=11) for SubCh, 26±5 ms (n=4) for Ach, and 11±2 ms (n=3) for carbachol. This sequence reflects the well known relationship between the open times of channels induced by these drugs at normal endplates[14,15] and at extrasynaptic membrane of hypersensitive fibres[12].

The results obtained so far, especially the pharmacological specificity, lead us to conclude that the observed conductance changes are indeed recordings of single-channel currents. They are consistent with the conclusions drawn from statistical analysis of endplate current fluctuations, and show that current contributions of individual channels are of the form of square pulses. In addition, analysis of areas under the peaks of histograms like Fig. 3*c* indicates that in our experimental conditions opening of individual channels is statistically independent, since the probabilities of zero, one, or two channels being open simultaneously follow—within the limits of experimental resolution—a Poisson distribution.

Recordings of single-channel currents finally resolves the third level of quantification in the process of neuromuscular transmission after the discovery of endplate currents and miniature endplate currents. It should facilitate discrimination between factors influencing the properties of single channels and agents creating or modifying different populations of channels.

We thank J. H. Steinbach for help with some experiments. Supported by a USPHS grant to Dr C. F. Stevens, and a stipend of the Max-Planck-Gesellschaft.

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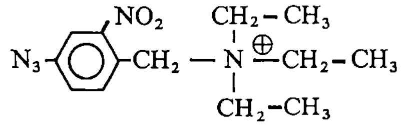
Selective inhibition of potassium conductance in node of Ranvier with a photoaffinity label derived from tetraethylammonium

BIOCHEMICAL investigation of membrane components involved in ion-specific conductance changes is difficult because of the lack of a functional assay for the components after membrane solubilisation. Proteins thought to participate in conductivity changes during synaptic transmission[1] have been isolated using a binding assay and affinity chromatography[2,3], because of their high affinity for a variety of ligands; but the connection between such receptor proteins and postulated ion channels remains to be shown. To isolate molecules forming sodium channels of the axon membrane[4–7], tetrodotoxin was used as ligand (binding assay $K_d = 3$ nM). The most efficient known blocking agent of the change in potassium conductance in nerve and muscle known is tetraethylammonium (TEA) but its affinity is too low ($K_d = 0.4$ mM (ref. 8)) for either of the above techniques. We have attempted to label radioactively those molecules associated with the voltage-dependent potassium conductance changes covalently *in situ* with the aim of recovering that radioactivity after solubilisation using classical fractionation procedures. We suggest that it is possible to block selectively and irreversibly potassium conductance in myelinated nerve fibres using a photoaffinity lable. This method may open the way for the isolation of the TEA receptor of excitable membranes, proposed as part of the potassium channel (if not the channel itself).

We applied 4-azido-2-nitrobenzyltriethylammonium fluoroborate,



$$N_3\text{-}C_6H_3(NO_2)\text{-}CH_2-\overset{\oplus}{N}(CH_2\text{-}CH_3)_3$$

which belongs to a class of photoaffinity labels developed by H.K.[9] and which was first used in the investigation of the structure of the acetylcholine receptor (F.H., P. Layer, H.K. and G. Bandini, unpublished) and the acetylcholine esterase (P. Layer, H.K. and F.H., unpublished). The label was synthesised using the same procedure described for the corresponding trimethylammonium derivative (P. Layer, H.K. and F.H., unpublished). Voltage clamp experiments were carried out with sensory and motor fibres of the sciatic nerve of the frog *Rana esculenta*[10,11]. The Ranvier node was continuously superfused with Ringer or the test solution as indicated in Figs 1–4. Photoaffinity labelling was induced by irradiation with an ultraviolet lamp Typ TL-900 (Camag, Switzerland; emission maximum 254 nm; lamp distance from fibre 5 cm). Without irradiation the photoaffinity label reversibly inhibited the potassium conductance (Fig. 1*c*), whereas the sodium conductance was not affected.

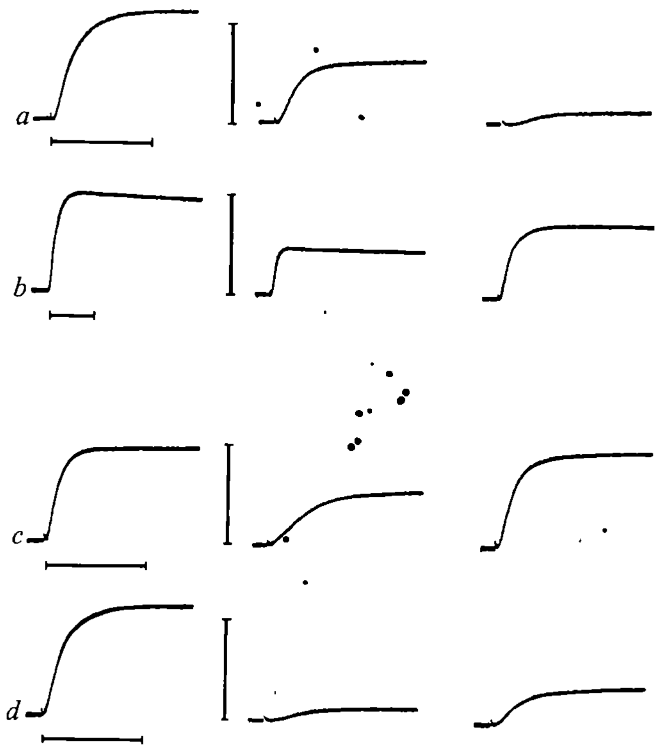
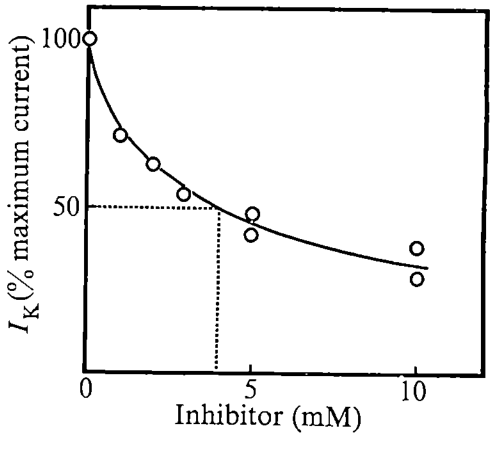


Fig. 1 Potassium current records during 140 mV depolarising pulses from a holding potential of −100 mV. Capacity and leakage currents were compensated analogically. Test node superfused with Ringer's solution (117 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$) plus tetrodotoxin (300 nM). Control, First record in each row. In each experiment photoaffinity label (1 mM) was added either to Ringer's solution (*a*, *c* and *d*) or to isosmotic KCl solution in contact with the cut ends of the fibre (*b*). *a* and *b*, Effects of photoaffinity label applied either externally (*a*) or internally (*b*), before (second records) or after (third records) application of ultraviolet light for 3 min. *c* and *d*, Reversibility for the experiment without (*c*) or with irradiation (*d*). Third record in *c* and *d* was taken 40 min after removal of photoaffinity label from Ringer's solution. Vertical and horizontal calibrations: 10 nA and 10 ms, respectively.

50% inhibition of the potassium current was obtained with 4 mM photoaffinity label (Fig. 2), indicating a much lower affinity than that for TEA ($K_d = 0.4$ mM (ref. 8)). During irradiation in the presence of the label the potassium current decreased markedly and rapidly (Figs 1*a* and 3), whereas the sodium current decreased only slightly (Fig. 3). In a typical experiment, we obtained after about 2 min irradiation an 80% decrease in potassium current and a less than 10% decrease in sodium current. After removal of the

Fig. 2 Dose–response curve for 4-azido-2-nitrobenzyltriethylammonium fluoroborate (without irradiation). Photoaffinity label was added to Ringer's solution used to superfuse test node. Temperature: 11 °C.



light source and washing the fibre with Ringer's solution, about 65% irreversible inhibition of the potassium current remained (Fig. 1*d*). Control experiments suggested that this irreversible inhibition arises by a photoreaction of the molecules bound to a receptor in the potassium channels and not from the action of photolytic products of the reagent. A solution irradiated before its application to the test node strongly inhibited the potassium conductance, but this inhibition was completely reversible. Exposing the test node to ultraviolet light in the absence of photoaffinity label showed that in our experimental conditions ultraviolet light alone had practically no effect on potassium conductance but a small effect on sodium conductance (Fig. 3*b*). These observations are in perfect agreement with similar observations by Fox[12].

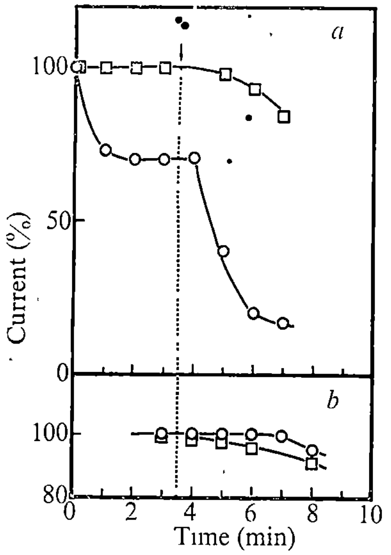


Fig. 3 Effects of photoaffinity label (*a*) and ultraviolet light (arrow) in the absence of photoaffinity label (*b*) on sodium (□) and potassium (○) currents. Ringer's solution with 1 mM photoaffinity label was applied 3.5 min before irradiation. The small effect observed on the sodium system may be explained in terms of the effects of ultraviolet light *per se* in the absence of photoaffinity label. Temperature 11 °C.

Photoaffinity label applied by diffusion from the cut ends of the fibre to the internal side of the nodal membrane caused, without irradiation, a similar selective inhibition of the potassium conductance (Fig. 1*b*). Surprisingly, irradiation with ultraviolet light had in this case the opposite effect to that in the case of external application of photoaffinity label (Fig. 1*b*). Instead of a further decrease there was an increase in potassium current, indicating that the binding site for the quaternary ammonium group is situated close to the internal opening of the potassium channel. According to this model, during irradiation in the case of application from the internal side of the axon, the 'reactive tail' of the photoaffinity label can react only with sites outside the channel—for example, with proteins from the axoplasm, the latter thereby being removed from the channel. When applied from the external side the label enters the channel far enough for a covalent attachment to sites inside the channel.

Analysis of the kinetics of potassium conductance activation after step depolarisation in the presence of photoaffinity label revealed a further difference between the internal and external site of the axonal membrane. When applied from the outside, without irradiation, the increase in potassium conductance was much slower compared with the control (Fig. 4*a*). After application from the inside the increase in potassium conductance was faster, and irradiation reduced it again to its original value (Fig. 4*b*). After the rapid increase in potassium conductance with some fibres a small inactivation of the potassium current was observed. This result is in agreement with experiments described by Armstrong[13,14], who observed an inactivation with internally applied benzyltriethylammonium and similar TEA derivatives.

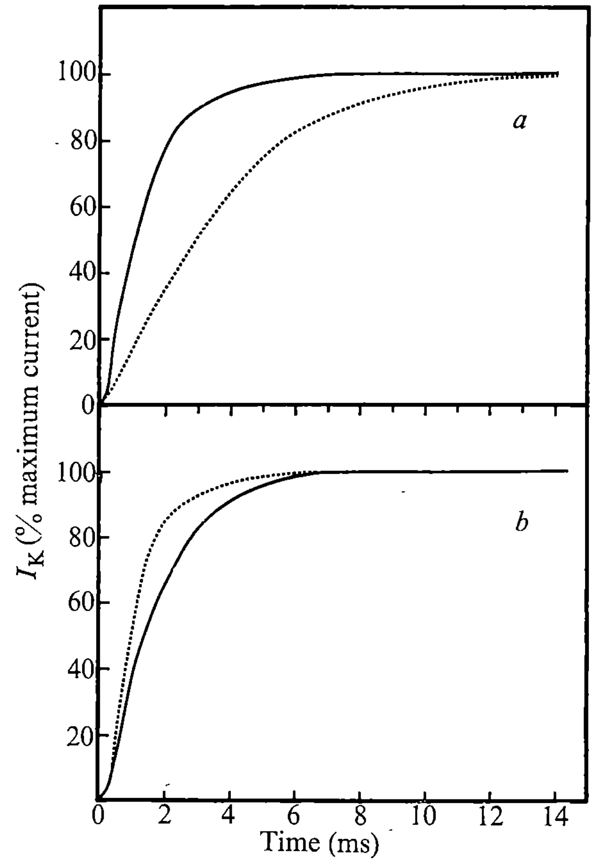


Fig. 4 Relative potassium current in the absence (solid line) or presence (dashed line) of photoaffinity label applied internally (*b*) or externally (*a*) without irradiation. *a*, 4 mM; *b*, 6.6 mM. Temperature, 11 °C.

One clear advantage of using our photoaffinity label is that its synthesis is straightforward, thus permitting radioactive labelling. Radioactive 4-azido-2-nitrobenzyltriethylammonium fluoroborate could be an important tracer for receptors associated with potassium channels.

This work was supported by the Deutsche Forschungsgemeinschaft SFB 138, the Fonds der Chemischen Industrie and the CNRS and DGST.

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Received February 10; accepted February 24, 1976.

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α Foetoprotein and serum albumin show sequence homology

α FOETOPROTEIN (AFP) is a major plasma protein of the foetus[1], where it is produced by the yolk sac and the liver[2]. In adults its concentration is extremely low[3] except in patients with tumours such as hepatomas and teratomas[4,5]. AFP has a molecular weight of 70,000, consists of a single polypeptide chain and contains about 4% carbohydrate[6]. The similarity of the chemical properties of AFP and albumin and the fact that their concentrations in the serum are high and inversely related[7] has led to the suggestion[8] that AFP may be a foetal counterpart of albumin. We have determined amino acid sequences of fragments of human AFP and found homology between these and the albumin sequence.

Human AFP was purified using immunoadsorbents[9]. The purity of AFP was checked with gel electrophoresis and immunological tests. The preparations used did not react with anti-normal human serum (Behringwerke) in immunodiffusion when tested at a concentration of 5 mg ml^{-1}. The procedures used and characterisation of the AFP preparations have been described in detail[5,9]. AFP was reduced, alkylated with iodoacetamide and cleaved with cyanogen bromide[10] Peptides resulting from the cleavage were separated on Sephadex G-100 (Pharmacia) equilibriated with 6 M urea and ·1 M propionic acid. Sequence determination was carried out on an automatic sequencer (Beckman, Model 890). The phenylthiohydantoin (PTH) amino acids were identified with gas chromatography and amino acid analysis following hydrolysis back to amino acids both in hydrochloric acid and hydroiodic acid.

Cyanogen bromide cleaves AFP to seven peptides detectable by gel electrophoresis. Their molecular weights are 23,000, 12,000, 9,000, 8,000, 6,500, 4,000 and 3,500 (ref. 11). The 23,000 (I) and 12,000 peptides (II) were purified and their N-terminal sequences determined. Comparison of these sequences with the albumin sequence[12–15] revealed significant homology between the AFP fragments and certain parts of the albumin molecule (Table 1) The percentage of same-sequence positions between the two peptides and human and bovine albumin was 37–45. Computer search for homology between the AFP fragments and known protein sequences[16] revealed no further significantly homologous sequences and clearly established the significance of the similarity with albumin (Table 2).

These sequence data clearly indicate that AFP and albumin have a common ancestor This is in line with their general similarity. Their chemical properties differ markedly only with respect to carbohydrate content. Albumin does not contain carbohydrate, whereas AFP is a glycoprotein[6].

Our data do not permit accurate estimation of the degree of homology between AFP and albumin. The comparison is based only on 59 residues (out of about 600). Furthermore, the N-terminal sequences of albumin[17] and AFP[18] do not show homology. This may be due to insertion of additional

Table 1 Homology between fragments from AFP and albumin[12–15]

K	N	F	G	T	R	T	F	Q	A	I	T	V	T	K	L	S*	Q	K	F	T	(K)†	V	(L)	F	T	Z	I	Q	AFP cyanogen bromide fragment I
Q	K	F	G	E	R	A	L	K	A	W	S	V	A	R	L	S	Q	K	F	P	K	A	E	F	V	E	V	T	Albumin (bovine) 202-230
Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	Q	R	F	P	K	A	E	F	A	E	V	S	Albumin (human) 203-231

S	Y	I	C*	S	Q	Q	D	T	L	S	N	K	I	T	E	S	S	K	L	T	T	L	E	R	G	Q	S	I	I	AFP cyanogen bromide fragment II
K	Y	I	C	B	B	Z	B	T	I	S	S	K	L	K	E	C	K	D	P	C	L	L	E	K	S	H	C	I	A	Albumin (bovine) 260-289
K	Y	I	C	Z	B	Z	B	S	I	S	S	K	L	K	E	C	K	E	P	C	L	L	E	K	S	H	C	I	A	Albumin (human) 261-290

*Assignment for serine as against cysteine should be regarded as extremely tentative due to the difficulty in distinguishing between the respective PTH derivatives using gas chromatography and back hydrolysis to amino acids.
† Tentative identification.

Table 2 Histograms of sequence comparison between AFP fragments and sequences in 732 other proteins*

Score	Peptide I	Homologous sequence	Peptide II	Homologous sequence
0	9,404		8,615	
1	16,411		16,551	
2	14,869		14,686	
3	8,658		8,565	
4	3,512		3,745	
5	1,163		1,258	
6	277		305	
7	95		85	
8	11		14	
9	0		2	Immunoglobulin K-chain–human Roy 172–201 Immunoglobulin μ-chain–human Ou 150–179
10	0		0	
11	0		1	Human albumin 261–290
12	0		1	Bovine albumin 260–289
13	2	Human albumin 203–231 Bovine albumin 202–230	0	

*Computer analysis carried out at the National Biomedical Research Foundation using existing sequence information[16]. The analysis compares the sequences of the AFP fragments with sequence fragments of corresponding length that are contained in the known sequences of 732 other proteins. The columns under peptides I and II indicate the number of sequence fragments with a given degree of similarity with the AFP fragments. This is expressed as the number of identical residue positions (=score) between the AFP sequence and the sequence with which it is compared.

sequence to the N-terminal of AFP or a deletion or post-synthetic modification in albumin. The fact that the pieces in the albumin sequence homologous with the relatively large AFP peptides are separated only by 30 amino acids, suggests rearrangement of these pieces in either one of the molecules.

Albumin binds a wide variety of different substances including fatty acids, drugs, and dyes[19]. AFP has been shown to bind certain hormones[20] and various dyes[21].

These similarities and the sequence homology suggest that albumin and AFP have similar functions as general carriers in plasma and perhaps in the maintenance of the osmotic pressure. In addition, AFP probably has special functions offering an advantage over albumin to the foetus. This may be connected with immunosuppression[22].

A detailed report on these results and further chemical characterisation of AFP will appear elsewhere.

We thank Mr R. Guyer, Mr Y. Kawanowa, and Ms V. Tiilikainen for assistance. Supported by the Finnish MRC, the Finnish Cancer Society, and the National Cancer Institute. Part of this work was carried out during the tenure of a Travel Fellowship awarded to E.R. by the International Agency for Research on Cancer.

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Quantitative adaptation of isoacceptor tRNAs to mRNA codons of alanine, glycine and serine

THE optimum efficiency of protein biosynthesis depends on the availability of aminoacyl-transfer RNAs (tRNAs) in the neighbourhood of active polysomes, among other factors The intracellular tRNA level is directly proportional to the composition of the amino acids incorporated into proteins being synthesised[1]. This continuous and selective adjustment takes place in prokaryotes and eukaryotes and in non-differentiated as well as in highly specialised cell systems[2]. We report here that such a proportionality takes place at the molecular level between the frequency of synonomous codons in messenger RNAs (mRNAs) and the distribution of the corresponding isoacceptor tRNAs. A correlation was found for the alanine, glycine and serine codons from viral nucleic acids and eukaroytic iso-tRNA sets fractionated on reversed-phase chromatography as well as for the preponderant tRNAs involved in the translation of fibroin mRNA in the posterior silk gland of the silkworm *Bombyx mori* L.

The overall ratio of different tRNAs must include a relationship between the frequency of synonymous codons in mRNAs being decoded and the respective set of iso-tRNA species. To verify this relationship, the distribution of synonymous codons for the predominant amino acids in mRNA and the anticodon distribution of the corresponding isoacceptor tRNAs must be known. The former can be obtained from a statistical study of a large number of mRNAs of known sequence in normal tissue or from the structural analysis of the main mRNA of a specialised tissue, whereas the latter is obtainable from the codon response of fractionated tRNA species and from sequences.

In this study the average frequency of synonymous codons in viral RNAs of which the sequence is partially or completely known is compared with the average distribution of the respective iso-tRNA species in several eukaryotes. According to the general analysis of Grantham[3] with coliphage RNAs, the distribution of frequencies for degenerate codon positions is far from random. We assume that the same distribution frequency occurs in mRNAs from

Table 1 Average distribution of iso-tRNAs from eukaryotes and frequency of synonymous codons for alanine, glycine and serine in viral RNAs and DNA

	Codon response of iso-tRNA	Mean value±s.d. (%)	Viral codons (%)	
	U		32	
Alanine	GCC	73.4±4.6	21	82
	A		29	
	GCG	26.6±4.6	18	18
	U		58	
Glycine	GG	65.8±1.9		79
	C		21	
	A		9	
	GG	34.2±1.9		21
	G		12	
	U		8	
Serine	AG	32.3±5.4		20
	C		12	
	U		26	
	UCC	56.3±2.4	14	57
	A		17	
	UCG	11.4±4.3	23	23

The proportions of iso-tRNA species are taken from mean values calculated from 15 independent elution profiles on reversed-phase chromatography for different eukaryotic cells. The codon response of each peak was measured by ribosome binding and the distribution obtained from the elution profile (for tRNA$^{\mathrm{Ala}}$, see refs 4 and 5; for tRNA$^{\mathrm{Gly}}$, ref. 4; and for tRNA$^{\mathrm{Ser}}$, refs 4, 6 and 7). For iso-tRNA$^{\mathrm{Ala}}$ species chromatographic data are provided from *Drosophila*[8], rat liver[9], rabbit liver[10], rabbit reticulocyte[11], calf muscle[12], human lymphoblasts[13] and brain[10]. The minor iso-tRNA$^{\mathrm{Ala}}$ species not only decodes GCG, but also GCA; it seems to be a mixture of a third species recognising GCU and GCC in yeast[5], as in prokaryotic organisms. A similar situation exists in the silkgland of *B. mori* L.[31]. For iso-tRNA$^{\mathrm{Gly}}$ species data were obtained from *Drosophila*[8], rat liver[9], rabbit reticulocyte[11], calf muscle and lens[12]. For iso-tRNA$^{\mathrm{Ser}}$ species data come from *Drosophila*[8], rat liver (refs 7 and 14, and H. Rogg, unpublished), rabbit liver[10], rabbit reticulocytes[11], hen liver[15], human lymphoblasts[13], rat adrenal cortex, glioblastoma C-6 (H. Rogg, unpublished) and beef brain[7]. The frequency of alternative codons in partially and completely sequenced viral RNAs was determined from structural data compiled by Barrell and Clark[16]. Additional data were kindly provided by Fiers before publication of the total sequence of bacteriophage MS2 RNA coding for the A protein[17] and the partial 3′-end sequence of MS2 RNA coding for viral RNA polymerase[18], and by Guilley[19] for the partial sequence of RNA from the tobacco mosaic virus coat protein cistron. Of 980 analysed codons, 35 comes from f2 RNA, 678 from MS2 (393 from A protein, 129 for coat protein and 156 for replicase), 32 from Qβ, 91 from R17 and 77 from TMV. We have also taken into account 67 codons from the G gene coding for spike protein of φX174 DNA[20]. The sampling for alanine, glycine and serine are respectively 82, 48 and 89.

Table 2 Distribution of iso-tRNAs, amino acid–tRNA ligases and codons for alanine, glycine and serine in the posterior silk gland of *B. mori* L. on day 8 of the fifth instar

	Alanine			Glycine			Serine			Refs
Amino acids of fibroin (%)	29			46			12			22
Amino acid–tRNA ligases (%)	31			42			13			29
tRNA (%)	26			42			16			24–26
Iso-tRNA (%)	3	6	17	20	7	15	5	9	2	27, 30
Anticodon (3′-5′)*	(CGU)	(CGG)	CGI	CCG	(CCN)	CCU*	(UCG)	AGI	AGC	31–33
Codons of fibroin mRNA (5′–3′)			GCU	GGU		GGA		UCA		23
Codons of fibroin mRNA (%)			29	27		19		12		—

*Presumed anticodon in parentheses.

†Estimated content according to the oligoribonucleotides analysis carried out by Suzuki and Brown[23] and to the amino acid composition of fibroin[22]. For calculating the linear correlation coefficient, we have taken both $tRNA^{Ala}$ species able to recognise GCU codon.

highly differentiated organisms The three tRNAs studied correspond to three main amino acids (alanine, glycine and serine) which account for 24±1% of the residues in common protein. The base in the 5′ position of the anticodon of their respective iso-tRNAs can be of four different kinds· inosine can recognise $XY\overset{U}{\underset{A}{C}}$ codons in $tRNA^{Ala}$ and $tRNA^{Ser}$, guanosine recognises $XY\genfrac{}{}{0pt}{}{U}{C}$ for $tRNA^{Ala}$, $tRNA^{Gly}$ and $tRNA^{Ser}$, uridine decodes $GX\genfrac{}{}{0pt}{}{A}{G}$ in $tRNA^{Ala}$ and $tRNA^{Gly}$ and cytidine UCG in $tRNA^{Ser}$ (see ref. 4 for review).

Table 1 shows the results of a statistical analysis of the composition of three iso-tRNA sets from 15 eukaryotic cell types after fractionation by reversed-phase chromatography. We have only retained the quantitative variations of the concentration of each isoaccepting species and *a priori* ruled out qualitative or structural modifications detectable with this type of chromatography. In spite of differences in resolution and some inversions in elution order due to experimental conditions, the variability in the number and proportion of the iso-tRNA species is small. It is thus possible to assign average values.

Table 1 also shows the frequency of synonymous codons for alanine, glycine and serine, which represent 22.4% of 980 codons analysed from viral RNAs and DNA φX174. Their distribution is non-random: XYU predominates. The correlation between the distribution of codons and the intracellular levels of the respective isoaccepting species corresponding to these codons is highly significant ($r = 0.93$).

It is also possible to correlate the iso-tRNA levels and codons frequency of a predominant mRNA in a specialised tissue such as the posterior silk gland of *B. mori* L The exponential accumulation of fibroin mRNA ($mRNA_F$) during the last larval instar up to 3.5% at the sixth day[21] leads to a massive biosynthesis of fibroin accounting for at least 75% of the proteins synthesised at the end of the secretion phase. Alanine, glycine and serine represents 87% of the total amino acids of fibroin[22]. Isolation and partial structural analysis of the 3′-exoguanylic oligoribonucleotides of $mRNA_F$ enabled Suzuki and Brown[23] to deduce the main codons for fibroin: GCU for alanine, GGU and GGA for glycine in a 1.4 : 1 ratio and UCA for serine. The distribution of glycine codons is markedly different from that in viral nucleic acids, for which the GGU : GGA ratio is 6 5 (Table 1). This distribution is sufficiently different to stimulate a distinctive composition of iso-$tRNA^{Gly}$ species in the posterior silk gland.

Several studies of the fibroin translational apparatus provide information about the intracellular levels of tRNAs during the fifth instar[24–28]. their cognate ligases[28–29], the changes of iso-tRNA species[27,30] and the nature of their anticodons determined either by response to codons[31] and to polynucleotides[32,33]; or directly analysed by sequencing; or by their modified nucleoside content (J.-P. Garel, unpublished). Table 2 clearly shows that the posterior silkgland iso-tRNAs match the peculiar distribution of their cognate codons in fibroin mRNA. ($r = 0.99$). Note that the ratio between the two main iso-$tRNA^{Gly}$ species (1.33) is different from that generally found (1.92, see Table 1).

The quantitative adaptation of iso-tRNA population to codon frequency of active mRNAs strongly suggests a coordinated regulation between the codon requirements of mRNA and the corresponding aminoacyl-tRNA species.

I thank W. Fiers, R. Grantham, D. Hentzen, J. Ofengand and H. Rogg for assistance.

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Bifurcated hydrogen bonds and flip–flop conformation in a modified nucleic acid base, gc^{6}Ade

FROM an analysis of X-ray diffraction data on modified bases of transfer RNA (tRNA) and their ribonucleosides, we have established that these molecules can be made to undergo, by suitable changes in *p*H, a reversible flip–flop conformational change from one stable state to another across a high potential barrier. We have also shown that positive charges adjacent to C–H bonds in the heterocyclic ring of nucleic acid bases polarise these bonds and enable them to take part in strong hydrogen bonds to appropriate acceptors.

The modified nucleoside *N*^{6}-(*N*-glycylcarbonyl)adenosine (g^{6}A), previously termed *N*-(purin-6-ylcarbamoyl)glycine ribonucleoside[1], is an analogue of *N*^{6}-(*N*-threonylcarbonyl) adenosine (t^{6}A), and has been isolated[2] from enzyme digests of unfractionated yeast tRNA. We have carried out X-ray crystallographic studies of these anticodon-adjacent hypermodified bases at or near acidic (*p*H ~ 0) and alkaline (~ 8.0) *p*H, and of their ribonucleosides at *p*H ~ 3.5. At alkaline *p*H tc^{6}Ade and gc^{6}Ade bases are anions and these were crystallised (*p*H ~ 8.0) and studied by us as the corresponding potassium salts. The molecular structure of the gc^{6}Ade anion as obtained from the crystal structure[3] of (gc^{6}Ade)$^-$K$^+$ displays two important features (Fig. 1); the base takes up the 'distal conformation'[4] and an intramolecular hydrogen bond is formed from the hydrogen on N(glycine) to the N(1) of adenine. These two features are found in the structure of (tc^{6}Ade)$^-$K$^+$.4H$_2$O (ref. 4) and also in the structures of the uncharged ribonucleosides t^{6}A (ref. 5) and g^{6}A (R.P., and G.B.C., unpublished). We discuss here the chemical implications of these two conformational features; their biological role has been suggested elsewhere[4–6].

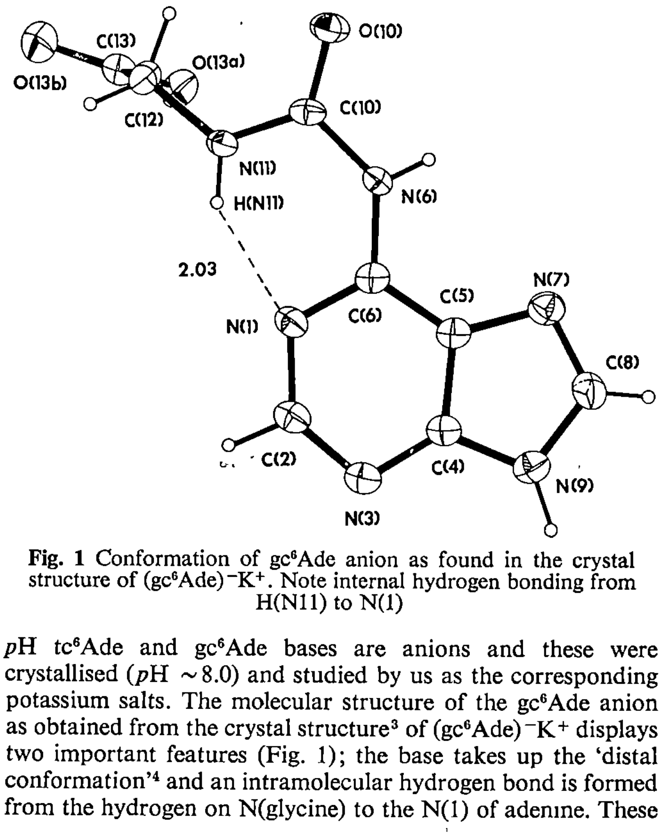

Fig. 1 Conformation of gc^{6}Ade anion as found in the crystal structure of (gc^{6}Ade)$^-$K$^+$. Note internal hydrogen bonding from H(N11) to N(1)

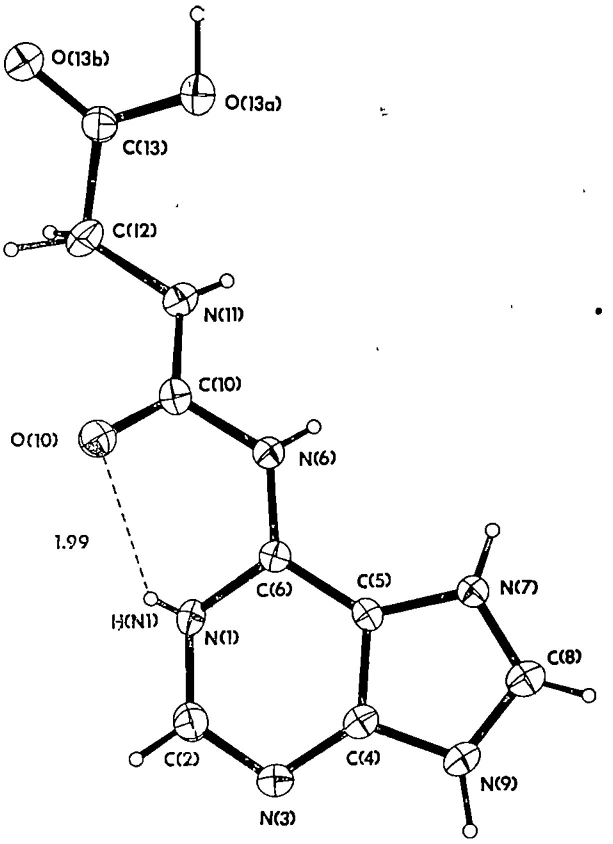

Fig. 2 Conformation of gc^{6}Ade dication as found in the crystal structure of (gc^{6}Ade)$^{2+}$ (2Cl)$^{2-}$. The protonation on N(1) has caused a change of conformation about the N(6)–C(10) bond giving rise to a different internal hydrogen bond, N(1)–H(N1) ··· O(10).

At acidic *p*H, the site of protonation for purine is N(1) although it has been argued[6] that it is N(7) or N(9). Monoprotonated adenine derivatives such as adenine hydrochloride hemihydrate[7], 3′-AMP (ref. 8), and 6-methyladenine hydrochloride[9], all show protonation on N(1). In gc^{6}Ade, N(1) is intramolecularly hydrogen bonded to H(N11) (Fig. 1); furthermore, the atoms N(11) and H(11) are held in this orientation by a series of conjugated covalent bonds, and are not free to move away from N(1). Consequently, it was of interest to determine in what conditions N(1) of gc^{6}Ade can be protonated. We therefore treated gc^{6}Ade with an excess of concentrated HCl solution to obtain crystals of the dihydrochloride derivative

Table 1 Table of bifurcated hydrogen bonds and weaker hydrogen interactions

D–H ··· A$_1$*	H ··· A$_1$	D ··· A$_1$	D–H ··· A$_1$	A$_1$ ··· H ··· A$_2$
D–H ··· A$_2$	H ··· A$_2$	D ··· A$_1$	D–H ··· A$_2$	(sum)†
N(1)–H(N1) ··· O(10)	1.99 Å‡	2.616 Å	132.1°	91.8°
N(1)–H(N1) ··· Cl(2$^{\text{i}}$)	2.49‡	3.141	136.1	360
N(7)–H(N7) ··· O(13b$^{\text{ii}}$)	2.22‡	2.847	153.5	84.3
N(7)–H(N7) ··· Cl(2$^{\text{iii}}$)	2 78	3.185	121 8	360
C(2)–H(C2) ··· Cl(1$^{\text{i}}$)	2.93	3 649	140.6	86.5
C(2)–H(C2) ··· Cl(2$^{\text{i}}$)	2 78	3.337	125.8	353
C(8)–H(C8) ·· Cl(1$^{\text{i}}$)	2.51‡	3.349	150.3	93.9
C(8)–H(C8) ··· Cl(2$^{\text{iii}}$)	2.88	3.260	108.7	353

*D is the donor and A is a possible acceptor atom.
†Sum = angle (D–H ··· A$_1$ + D–H ··· A$_2$ + A$_1$ ··· H ··· A$_2$).
(i) $1-x, -y, 1-z$; (ii) $-x, \frac{1}{2}+y, -\frac{1}{2}+z$; (iii) $1-x, 1-y, 1-z$.
‡Considered as hydrogen bonds; the other contacts are considered as weaker hydrogen interactions.

of gc^{6}Ade. These crystals are monoclinic, space group $P2_1/c$ with unit cell parameters: $a = 13.069(3)$ Å, $b = 9.878(1)$ Å, $c = 12.866(4)$ Å, $\beta = 134.27(1)°$, $D_{obs} = 1.65$ g cm^{-3}, D_{calc} (for $C_8H_{10}N_6O_3Cl_2$) = 1.62 g cm^{-3}, $Z = 4$, temperature = 22±3 °C, λ(CuKα) = 1.54051 Å. Using diffractometer data (2,582 reflections to the limit $2\theta = 160°$ for CuKα) the crystal structure was solved, and refined by the least-squares method to $R = 0.056$. Detailed correction for absorption was carried out. The hydrogen atoms were located from electron-density difference maps and their parameters refined by the least-squares method.

The structure analysis shows (Fig. 2) that the molecule is a dication with protonation on both N(1) and N(7) or N(9). Concomitant with the protonation is a change in the conformation of the molecule, a *cis–trans* isomerisation across the N(6)–C(10) bond stabilised by the formation of an intramolecular hydrogen bond N(1)–H(1) · · · O(10). Thus, the change in *p*H to the acidic side 'flipped' the molecule from one stable conformation to another stable one across a high potential barrier; the original conformation can be restored by increasing the *p*H. The basic pK_a of gc^{6}Ade measured by us is 2.6, compared with a pK_a of 3.8 for adenine. It seems that the hydrogen bonding causes an hysteresis effect; the titration curve in one direction is not reproduced on back titration, as in the cytosine moiety of DNA[10]. The double protonation of the adenine ring has caused considerable delocalisation of the charge in the adenine ring, and has highly polarised[7,11] the N(1)–H, N(7)–H, C(2)–H and C(8)–H bonds so that they take part in bifurcated hydrogen bonds—or weaker bifurcated hydrogen interactions (Table 1). The C(2)–H and C(8)–H hydrogens have no such contacts in the neutral and anionic forms of gc^{6}Ade. In all these bifurcated bonds and weaker bifurcated interactions, the hydrogen atom lies close to the plane through the donor and the two acceptor atoms[12,13].

We thank Dr D. Harker for discussions and the US National Cancer Institute for financial support. Reprint requests to CCR address.

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Balance between swelling pressure and collagen tension in normal and degenerate cartilage

ARTICULAR cartilage contains a high concentration of acid glycosaminoglycans (GAG), reaching 6% by wet weight and associated with fixed charge densities up to 0.2 mEq g^{-1}. This leads to considerable swelling pressure within cartilage, due to, first, the strongly non-ideal osmotic pressure, characteristic of polymer solutions, which increases sharply with concentration and, second, the ionic contribution, in accordance with the Gibbs–Donnan equilibrium. Ogston and Wells[1-3] have estimated the values of these two components and I have calculated them from my experimental data on cartilage[4,5]. The two components of swelling pressure are approximately of the same order of magnitude and can reach values as high as 1.7 kg cm^{-2} (refs 4 and 5). Since normal cartilage does not swell in solution, even when it is removed from the joint and cut into thin (250 μm) slices (lowest curve, Fig. 1), this implies that its high swelling pressure must be counteracted by considerable elastic forces within the collagen fibre network. It has been known for some time that the concentration of GAG gradually increases from the articular surface to the deep zone (for example, refs 6 and 7). A typical variation of total GAG content with depth, measured as fixed charge density, is shown in Table 1. I now suggest that this particular profile is adapted to the physiological function and mechanical properties of cartilage.

Table 1 Ionic components of swelling pressure corresponding to different values of fixed charge density

Distance below articular surface (mm)	*Fixed charge density (mEq per g of wet tissue)	†Ionic component of swelling pressure (kg cm^{-2})
0	0.05	0.16
0.25	0.10	0.45
0.75	0.15	0.8
1.75	0.18	1.7
2.25	0.20	2.1

*The figure for the surface concentration of charged groups was obtained by X-ray probe analysis[17]. The other figures were obtained by the tracer cation method[11] and verified by chemical analysis[5].

†The values of the ionic component of swelling pressure were calculated using Na$^+$ and Cl$^-$ partition data obtained experimentally for slices of human femoral head cartilage (my work with W. D. S. Freeman, in preparation), and a formula given by Katchalsky *et al.*[18], to estimate the fraction of osmotically 'active' counterions. Similar values were obtained when a procedure suggested by Wells[12] and based on Manning's approach[20] was used in the calculations.

First, it must be remembered that a high GAG content is essential to the load-bearing requirements of joints for it confers on the tissue low hydraulic permeability[8] and high swelling pressure[1]. It thus serves both to limit the amount of liquid lost when cartilage is loaded in compression (and hence the resulting creep), and to ensure the speedy "recovery" of cartilage during the "off-load" intervals in the walking cycle[4-5].

If, however, the GAG concentration were uniformly high throughout the cartilage thickness, right up to the interface with synovial fluid, there would be a steep osmotic pressure gradient across this interface. The local tensile stresses in the collagen fibre network oppose the local swelling pressure gradients at any point within the tissue, and so, at the surface, the collagen fibres would have to be under exceptionally high tension to oppose the very high osmotic pressure gradient which would be present there. In reality, the GAG concentration at the surface is at its lowest, rising to a broad peak in the middle zone. Table 1 shows the values of the ionic component of swelling pressure as a function of distance from the surface. A fairly even gradient of swelling pressure is observed in the tissue, with only a small fraction of the maximum pressure at the interface with synovial fluid. This grading of stresses, however, seems to be designed more as a protection against long term fatigue, since collagen fibres in normal cartilage do not disrupt instantaneously or allow any measurable swelling when thin slices from whatever zone are exposed to solution (as mentioned above and shown in Fig. 1, lowest curve).

In contrast, degenerate cartilage swells easily. It has long

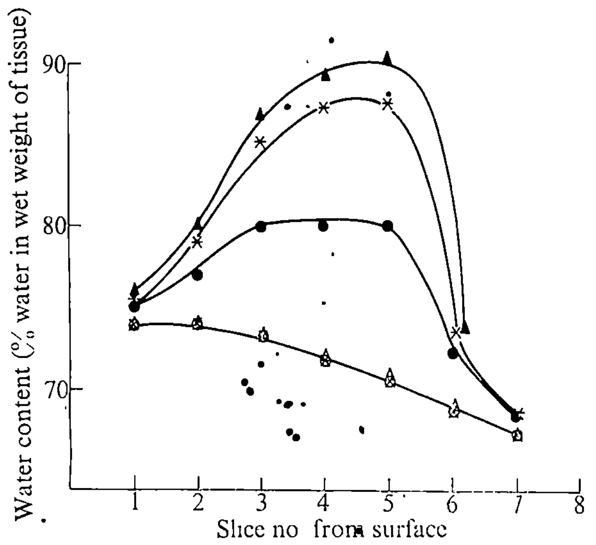

Fig. 1 Hydration in normal and degenerate cartilage. The moisture distribution in the chunk was obtained by slicing the latter on a freezing microtome into 250-nm slices and weighing these directly The subsequent swelling of the slices was determined by immersion in appropriate solution and weighing again after equilibration. Degenerate cartilage: ●, as cut; ▲, after soaking in 0.015 M NaCl; *, after soaking in 0.15 M NaCl Normal cartilage; ○, as cut; △, after soaking in 0.015 M NaCl; ×, after soaking in 0 15 M NaCl.

been known that one of the symptoms of cartilage degeneration is increased water content (for example, refs 9–11). This may seem surprising, for cartilage degeneration is usually accompanied by a loss of glycosaminoglycans. Few plausible explanations have been suggested for this paradox. I have suggested[11] that a failure in the elastic restraint of the collagen network enabled fibrillated tissue to swell to a higher degree of hydration in spite of the relatively low osmotic pressure of its decreased GAG content. I now present data in support of this hypothesis.

Figure 1 shows a profile of variation in water content with distance from the articular surface for both normal and mildly degenerate cartilage (my work with M. Venn, submitted for publication). In the normal cartilage, the water content seems to be little affected by the local GAG content. In degenerate specimens there is a distinctly different pattern, with a maximum in the middle zone, which I interpret as follows If the collagen network is partly damaged, its restraining effect on the swelling will be diminished and the hydration profile in cartilage should parallel its GAG content (which is known, in mildly degenerate tissue, to be rather low at the surface but still reasonably high in the middle zone (refs 11 and 12)). An intact collagen network is often still preserved in the deepest zone and thus there is no possibility of swelling in this region.

I further found that when a full depth chunk of surface-fibrillated cartilage is cut into 250-μm parallel slices, and these are immersed in buffered physiological saline (0.15 M NaCl), considerable swelling occurs in the slices from the middle zone, compared with their original water content (Fig. 1). This is consistent with the increased osmotic stress in these slices when exposed directly to isotonic saline solution, compared with the situation within the chunk. The swelling of middle zone slices was even more pronounced when they were immersed in a hypotonic solution (0.015 M NaCl), since the ionic component of the pressure differential was thus further increased (Fig. 1).

These results point to the fact that increased hydration in degenerate cartilage is due to damage of the collagen network and not, as suggested[13], to an increased fraction of so-called "bound water".

Finally, I wish to draw attention to a further implication of the above findings. *In vivo* the loss of GAG from the surface in an early stage of degeneration must, by this reasoning, lead to a steeper swelling pressure gradient in the middle zone, which should in turn lead to a greater stress on the collagen network there. It seems a reasonable supposition that fibres under tension are more liable to scission, whether by mechanical or enzymatic agents. I therefore suggest that once the surface layer of cartilage begins to lose its GAG, a self-perpetuating destructive process ensues in which successively deeper layers are exposed to gradual collagen disruption through GAG depletion.

It is still not known whether the primary event which leads to cartilage degeneration is glycosaminoglycan loss brought about enzymatically, with the damage to the collagen network following[14], or whether mechanical fatigue[15] is the direct cause of fibrillation, with the damaged collagen network subsequently enabling the proteoglycans to escape from the articular surface. But the observation that both in the early stages of an experimentally induced osteoarthrosis in the dog[16], and in some visually intact specimens of human osteoarthrotic cartilage removed for joint replacement (my work with M. Venn, submitted for publication), the water content is invariably increased, while the GAG levels remain normal, suggests (because of the casual association put forward here between the swelling of cartilage and a damaged collagen fibre network) that collagen fatigue and breakdown may be the earliest in a destructive chain of events.

Whatever the nature of the first step, it is obvious that early swelling of cartilage must also lead, through dilution of GAG in the tissue, to increased hydraulic permeability and decreased osmotic pressure[4], and thus must create an unfavourable mechanical environment in subsequent stages of the self-destructive process outlined here.

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Growth of algal symbionts in regenerating hydra

BETWEEN 10 and 20 photosynthetic algae are maintained per digestive cell in green hydra, the freshwater polyp. In normal conditions the number of symbionts maintained within the host remains stable—the algae do not overgrow the host nor do the animals outgrow the algae[1]. In experiments described here we found that when the upper two-thirds of a hydra was removed, there was a pronounced increase in the number of algae per digestive cell in the remaining peduncle of the animal. The observed increase in algal numbers was not a function of starvation and could be abolished by grafting a head, from a donor animal, on to the peduncle. We believe that our experiments are the first to show that the reproduction of symbiotic algae may be influenced by the host and that the presence of a head, thought to be responsible for maintenance of polarity and other morphological phenomena in hydra[4], may also influence the reproduction of symbiotic algae.

We used a variant of the green hydra, referred to as the English strain. Animals were reared and maintained under a 12 h light/12 h dark photoperiod and fed *Artemia nauplii*[3]. Five experimental organisms were macerated together and a sample of the resulting cell suspension was examined under a compound microscope. The algae in 150 randomly selected digestive cells were counted[1,4].

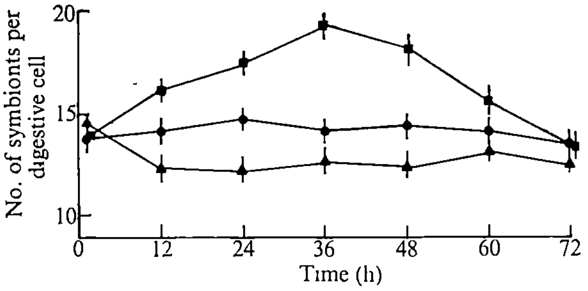
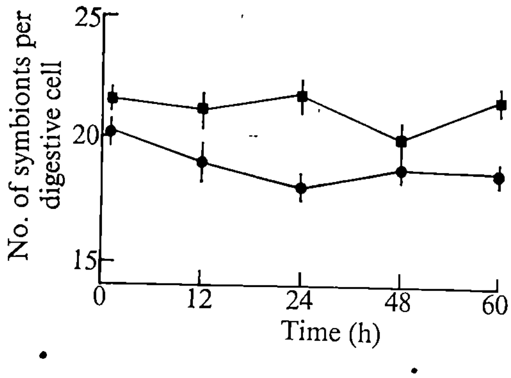


Fig. 1 Average number of algae per digestive cell from peduncle region of hydra at various times after removal of the upper two-thirds of the animal. Vertical bars show the standard error, $n = 150$ digestive cells. ■, Regenerating peduncle: analysis of variance indicates significant difference between points ($P < 0.001$). ▲, Peduncles removed from unfed animals at time of counting: analysis of variance indicates no significant difference between points ($P > 0.10$). ●, Peduncles with grafted heads: analysis of variance indicates no significant difference between points.

In our first experiment we removed the upper two-thirds (head, gastric and budding regions) of a group of hydra. At 12-h intervals after amputation, the algae per digestive cell were counted in the regerating peduncles. Green hydra normally maintains about 15 algae per digestive cell in the peduncle region. The regenerating peduncles did not eat throughout the experiment. Thus for controls, peduncles freshly isolated from unfed, intact animals were also analysed. Figure 1 shows that in regenerating peduncles the number of algal symbionts per digestive cell increased 25% from about 14 to 20 within 36 h of amputation of the upper two-thirds of the animal. After this increase in symbionts there was a decline in numbers per cell until the original level was established at 72 h, when the peduncle had regenerated the head and could feed. No change in algae per cell was observed in the control animals (Fig. 1*b*).

Fig. 2 Average number of algae per digestive cell from gastric region of hydra. Vertical bars show the standard error, $n = 150$ digestive cells. ■, Gastric region from animals after amputation of peduncle: analysis of variance indicates no significant difference between points. ●, Number of algae per digestive cell of gastric region removed from unfed animals at time of counting: analysis of variance indicates no significant difference between points.



To determine the possible influence of the head region on the growth of algae in the peduncle, we grafted heads from donor animals to the amputated peduncles. As before, these organisms were not fed and were analysed every 12 h. As Fig. 1 shows, there was no increase in the number of algae per digestive cell in peduncles with grafted heads. Furthermore, there was no change in the number of algae per digestive cell in the upper gastric region (with heads) after amputation of peduncles (Fig. 2). These cells in the upper region normally contain approximately 20 symbionts.

Apparently the presence of a grafted head region prevented or inhibited the algal increase observed in the absence of the head region. It has been suggested that the head region is the site of morphogenetic substances which maintain the normal polarity and organisation of the animal[5]. Our results suggest that factors associated with the head inhibit algal reproduction in the peduncle also. These factors might act directly on the reproductive processes of the algae or indirectly through processes in the host's digestive cells in which the symbionts reside. When amputated, the peduncle tissue is removed from the influence of the head and the symbionts, now released from inhibition, begin multiplying.

It is not clear how the number of algal symbionts is reduced after the initial increase (Fig. 1). A possibility is that subsequent host cell division causes parcelling of the symbionts among the daughter digestive cells. The relationship between the head and animal cell division in the peduncle is unknown.

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Received December 31, 1975; accepted March 16, 1976.

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Errata

In the article "Involvement of DNA in resistance of potatoes to invasion by *Phytophthora infestans*" by M. Yamamoto and K. Matsuo (*Nature*, **259**, 63; 1976) the legends to Figs 1 and 2 were transposed.

In the article "The complete nucleotide sequence of bacteriophage MS2 DNA : primary and secondary structure of the replicase gene" by W. Fiers *et al.* (*Nature*, **260**, 500; 1976) Figs 1 and 5 have been transposed.

In the article "Brain immunoreactive gonadotropin-releasing hormone in Huntington's chorea and in non-choreic subjects" by E. D. Bird, S. A. Chiappa and G. Fink (*Nature*, **260**, 536; 1976) page 538, lines 8–9 In the female this could not account . . . should read In the female this could account. . . .

reviews

Enlightening chemistry

R. V. Jones

Philosophical Chemistry in the Scottish Enlightenment: The Doctrines and Discoveries of William Cullen and Joseph Black. By A. L. Donovan. Pp. x+343. (Edinburgh University: Edinburgh, January 1976). £7.

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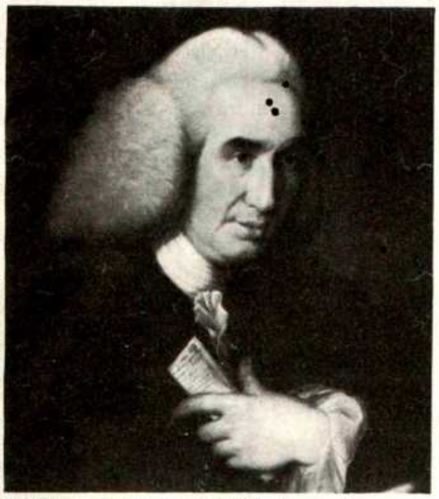
William Cullen (1710–90), from the original by William Cochrane

Photo: Royal College of Physicians of Edinburgh

THE Scottish intellectual enlightenment in the eighteenth century is rightly attracting attention among current historians of science in the US. A few months ago we had from Richard Olson an interesting book on *Scottish Philosophy and British Physics 1750–1880* (*Nature*, **258**, 285; 1975) on the effects of the Scottish common sense tradition on the development of British physics. And now we have from A. L. Donovan of the University of West Virginia a study of the interaction of two great Scots chemists of the enlightenment, William Cullen (1710–1790) and his pupil Joseph Black (1728–1799).

Donovan gives a good picture of the intellectual state of Scotland and its universities in the eighteenth century, and one of his aims is to demonstrate that "the advancement of scientific knowledge was a matter of both philosophical and practical concern in eighteenth century Scotland". He was drawn to study the question of how Black came to discover 'fixed air' (carbon dioxide) and this in turn led him to investigate William Cullen, with whom Black worked in Glasgow after graduating in arts and medicine. This was at about the time when, thanks largely to Cullen, chemistry was becoming a university study in its own right instead of being merely part of natural philosophy on the one side or medicine on the other.

If anything, Black was more of a natural philosopher, as shown by his work on specific heat and latent heat; but it was the medical need to find a solvent for renal stones that led him to recognise that chalk contained 'fixed air' which could be released as a gas by heat or acids, but which could also be transferred from one substance to another in solution without being released as effervescence. Donovan has gone to much scholarly trouble to trace this development in Black's mind—perhaps all the more interesting because it occurred against the background of Black's acceptance of the Phlogiston theory which Lavoisier's work later forced him to abandon.

Joseph Black (1728–99) by David Martin

Photo: University of Edinburgh

Black's influence, of course, extended beyond chemistry, and the last chapter of the book surveys his interaction and friendship with John Robison and James Watt, to whom Black's concept of latent heat was especially relevant. And when Watt invented the condensing steam engine Black (who in later life weighed every golden guinea that his students paid him in fees to check that they had not been cheating by filing the coins) lent him one thousand pounds to develop the engine.

Donovan has produced a useful, detailed, and well annotated study of an important phase of the Scottish scientific heritage. ☐

Immunology in vogue

A. J. S. Davies

The Immune System: A Course on the Molecular and Cellular Basis of Immunity. By M. J. Hobart and Ian McConnell. Pp. xxiii+357. (Blackwell Scientific: Oxford and London; Lippincot: Philadelphia and Toronto, 1975.) £5.00

SURELY the function of a book is communication between the author and reader. In this respect far too many contemporary scientific texts are deficient. They seem to operate on the assumption that the message is there—if you look hard enough. Aggregates of papers each by a different author with little editing or editorial comment are particularly prone to this elitism. It is then a happy event to find this book. It arose out of the course in advanced immunology sponsored by the Department of Immunology at the Royal Postgraduate Medical School and contains an amplification of the lecture notes. There has, however, been much useful editorial grading and cross-linking.

There are four major sections, each containing several chapters, on immunochemistry, immunobiology, immunogenetics and immunopathology. Each section has a chatty editorial introduction by no means as prolix as their designation 'preambles' would suggest. The range of topics is considerable although by no means comprehensive. In the immunopathology section for example, there are chapters on the so-called tumour immunology, immunodeficiency diseases, and autoallergic conditions but nothing on parasitic disease.

The illustrations, with the exception of one or two rather indeterminate electron micrographs (for example, on p60) are first class and provide an important visual element to the verbal complexities of contemporary immunology.

Following the present vogue, the book is predominantly concerned with cells and molecules and, with the exception of the illustrations of T and B areas on p210, there is hardly any histopathology. The approach of the synthesist in immunology is long overdue, and although there is a good chapter on the structure and function of the lymphoid system by McConnell this is not enough for my taste. A section on immunopathology can hardly be complete with no consideration of reactive changes in lymphoid organs. The book as a whole is so nearly comprehensive as to make consideration of remedy of these minor deficiencies worthwhile.

The book throughout is very readable, and the intention of the editors to communicate with their readers is made clear and is resoundingly successful.

In an age when new books tend to be greeted with a groan by overladen minds here is one for your personal perusal. Don't leave it to the library—get one yourself, it's a splendid book. ☐

Methodical investigation of dynamic systems

Introduction to Mathematical Control Theory. (Oxford Applied Mathematics and Computing Science Series.) By Stephen Barnett. Pp. viii+264. (Clarendon: Oxford; Oxford University: London, 1975.) £5.75 net.

THERE has recently been a shift of interest in control theory from engineering towards applied mathematics. Although this reflects growing awareness of the range and depth of mathematical problems which have been formulated in terms of modern control theory it also sadly reflects a growing disillusionment on the part of the professional engineer at the failure of control theory to realise but a small fraction of what was promised a decade ago. In recent years there has been a significant return to linear system concepts to formalise the techniques necessary for analysing systems in which the numbers of control variables are large. This has led naturally to the use of state vectors and matrix system operators to describe the dynamic performance of systems. It is here that the first difficulties arise since not only are matrix equations often difficult to manipulate, but they do not provide a physical insight into the nature of the control problems which the semigraphical older methods of control theory provided.

There clearly is a need for basic textbooks which can introduce the newer aspects of control theory and, if possible, link them with more established techniques. The audience would include applied mathematicians new to the control field, as well as students and practicing engineers. Stephen Barnett's book roughly supplies this need. Engineers will not find it an easy book to read since matrix algebra at this level is a demanding discipline. This is not made easier by the concise treatment given to many theorems but this is unavoidable in a work of this length. The reader will be struck, however, by the symmetry and compactness of the general theory which is beginning to evolve. The mathematician will find unusual outlets for studies in stability theory and the calculus of variations.

Although the book is by no means exhaustive in its range, it does cover a basic related set of topics and prepares the ground for further study. There are numerous worked examples and exercises but, as in most control textbooks, these describe problems which are almost trivial from an engineering point of view. It is argued that once the concepts have been mastered the extension to worthwhile problems is straightforward and is considerably aided by computational methods. It is of course here that the rift between theory and practice occurs. The formulation of real problems in terms of linear dynamic models, or often in any mathematical form, is difficult if not actually meaningless. Solutions, except in terms of empirical effort, are expensive to obtain and may then be impractical to realise. In spite of all this, sensibly applied control theory has much to offer in providing a framework for the methodical investigation of dynamic systems. This small but scholarly book will help to build such a framework.

J. M. Nightingale

Showing the flag

Geodynamics Today: A Review of the Earth's Dynamic Processes. (Sponsored by the British National Committee for Geodynamics for the Inter-Union Commission on Geodynamics.) Pp. 197. (The Royal Society: London, 1975.) £2.75 UK; £2.85 Overseas, including postage.

THE Geodynamics Project (1972–1977) is a loosely-coordinated international program of research into the dynamics and dynamic history of the Earth with emphasis on phenomena affecting surface and near-surface processes and structures. In 1973 the US Geodynamics Committee published, at no charge, a highly regarded and more or less definitive 235-page report on the scope and objectives of the Project. Soon after it appeared, the British National Committee for Geodynamics decided that something more than a preliminary British statement (published by the Royal Society in 1971) would be appropriate; and the result, published more than halfway through the Project, is *Geodynamics Today.* It is a sad reflection that in inflation-ridden 1970s Britain one has to pay to view the flag.

And the flag is a bit tatty at that. In this loosely-coordinated non-international program of 23 short state-of-the-art reviews on geodynamics some authors have dealt in generalities, others have resorted to detail; some have emphasised the past-to-present, others have concentrated on the present-to-future; some have included references and/or diagrams, others have gone for words only; some have put their heart into it, others have not. The result is a hotchpotch in which, the overriding geodynamic theme apart, subjects are linked only by virtue of the active or passive interest of the British scientists concerned. Nevertheless, the topics cover a wide range of geodynamic problems; and if no-one will wish to read everything, there is something here for almost everyone.

It is impossible to mention every subject covered and difficult to select a few. But of particular interest, because they are concerned with expanding themes of general consequence, are J. Sutton on the geodynamics of the Precambrian, E. R. Oxburgh on plate collisions past and present, I. G. Gass and others on the origin and emplacement of ophiolites, W. S. Fyfe on the influence of the hydrosphere on magma and D. H. Tarling on pre-Mesozoic palaeomagnetism. Also worthy of note, but for a different reason, is the article by N. N. Ambraseys, one of the few people to recognise the importance of historical seismicity studies, especially in the scientifically unpopular Mediterranean region.

If one general point emerges from this collection, or part of it, it is that British Earth scientists of various persuasions are particularly well placed, by inclination and experience, to lead the assault on the problems of pre-Wegenerian geodynamics. It is to be hoped that the money will be available for them to do so.

Peter J. Smith

Lunar Mineralogy. By Judith W. Frondel. Pp. x+323. (Wiley-Interscience: New York and London, October 1975.) £11.20.

THE ending of the Apollo and Luna missions marks a significant turning point in the development of lunar science Over the past five years efforts have concentrated on frantic reconnaissance and sample analysis in preparation for future missions, but now the pace has changed and problem-orientated studies and a more thoughtful evaluation of published data seem to be the most obvious of immediate future developments. In this situation the appearance of a book of this type is particularly valuable. About 850 pounds of rock were recovered in the Apollo programme, but it is important to remember that of this only about 10% has been extensively examined; approximately 20,000 individually identified samples remain available for future study.

This book has grown from two editions of a *Glossary of Lunar Materials* which the author prepared for distribution by NASA to principal investigators of the Apollo programme. It is arranged in the manner of Dana's *System of Mineralogy* and summarises all lunar mineralogical data published up to and including the Fifth Lunar Science Conference in March 1974. The author's task has been aided by the Earth's satellite, for rich repositories of rare and spectacular minerals such as pegmatites, true granites and hydrous vein deposits are conspicuously absent in the Moon; the rocks remain in a highly reduced state and there is a marked depletion in elements more volatile than iron. It is not surprising therefore to learn that only 60 valid and 14 tentatively identified mineral phases have so far been reported compared with about 2,200 on Earth, and there are no lunar diamonds.

Individual minerals are described in eleven systematic chapters; the treatment here is concise but adequate, and each section gives a good account of how particular minerals occur in lunar rocks. There are useful tables with selections of mineral analyses, photomicrographs wherever possible, and abbreviated references The wisdom of including here abbreviated optical and X-ray data on minerals such as olivines is questionable; the photomicrographs and specimen photographs are of a high quality, and the eye is captivated by the beautiful scanning electron microscope photographs of iron crystals deposited in vugs. The systematic chapters are preceded by an excellent 16-page introduction which summarises the history and petrology of the lunar surface, this section can be thoroughly recommended to amateurs. With these in mind, however, it is disturbing to read on page 7 that "Total and partial melting, a relatively insignificant feature on Earth, is important in the petrogenesis of the lunar highlands".

The author and publishers are to be congratulated on the high standard of production The book will be a standard reference for many years to come, and will be particularly valuable for those who have not worked on lunar minerals. The main text is supported by a bibliography, simple index and appendices which list the samples mentioned in the text and explain the sample-numbering system.

I. D. Muir

Lunar science

Lunar Science. A Post-Apollo View—Scientific Results and Insights from the Lunar Samples. By S. R. Taylor. Pp. xix+372. (Pergamon: New York and Oxford, 1975.) $16.50, £6.90.

THE technological *tour-de-force* in landing men on the Moon in the six missions Apollo 11, 12, 14, 15, 16 and 17 and the drama of Apollo 13—in particular the extreme sophistication of the systems control—has perhaps overshadowed the scientific achievements in the study of the returned lava, breccia and soil samples and the observations from experimental apparatus, some still functioning on the Moon. How appropriate it would be in this bicentennial year of the US if NASA produced a history of this unprecedented scientific project: by what alchemy did imaginative planning emerge from committees of scientists and administrators in Washington. But in some ways the very triumph of the scientific collaboration has been buried in vast conference proceedings; and it has been left to Dr Ross Taylor to give a most admirable answer to the question insistently asked "What really did this project discover about the Moon?".

Many of the conclusions reached or reinforced by evidence brought back by the Apollo project are set out clearly by Dr Taylor, and the book is a remarkable one: no scientist could fail to be fascinated and even those most learned about the Moon will learn something more. One miracle, however, was not achieved by the planners of the Apollo project: in spite of all attempts it has not entirely succeeded in bringing about an easy communication between petrologists and geochemists on the one hand and geophysicists on the other. In the many lunar conferences once the former begins talking about ferropseudobrookite and hedenbergite—ferrohedenbergite, the eyes of the latter become glazed; and when the latter begin speaking of electromagnetic transfer functions and magnetic Reynolds numbers, the conference room empties noticeably. In his treatment of the physics of the Moon, mainly in chapter 6 (only 29 pages), Dr Taylor falls from the high standard elsewhere.

Yet it was in this field that three of the most surprising and unexpected discoveries were made—positive anomalies in gravitational field occurring over the circular maria, remanent magnetisation of the returned specimens, along with magnetic anomalies in the lunar crust and moonquakes occurring predominantly at apogee and perigee. Dr Taylor does not treat these discoveries—fundamental to any understanding of the Moon's interior and its evolution—in the depth they deserve. For example in lunar magnetism he relies on a review, which he says provides "appropriate skeptical comments on current theories." Although some ideas which have been suggested to explain this unexpected finding are plausible—Velikovsky's prediction that the lunar surface was magnetised in 3,000 BC—some theories merit serious consideration. But some inconclusiveness is only to be expected when a new phenomenon is discovered. Observations that can be immediately understood in terms of currently accepted orthodoxy are not of so much interest as those which open up new approaches.

The fundamental significance of the non-hydrostatic figure of the Moon is ignored and thermal convection occurring by solid-state creep in its interior is not mentioned. The essential requirement of a theory of mascons—that is, that extra mass, whether lava or not, must be brought by lateral transport into the circular mare area—is not made clear. The implications of the remarkable correlation between the occurrence of moonquakes and the tides, and the analogous effect with regard to the enigmatic lunar transient events, are also not brought out.

S. K. Runcorn

obituary

Professor W. Ehrenberg, who died on November 19, 1975 at the age of 74, was born and brought up in Berlin. When a boy, he fell a victim to poliomyelitis which left him severely handicapped, but he never allowed this to impede his work in experimental physics, to which he transferred his main interest immediately after gaining his Ph.D. from Heidelberg in 1924. In 1926 he published with H. Mark and G. von Susich some now classic papers in which the natural width of the K X-ray lines was demonstrated and the two-crystal X-ray spectrometer proposed and analysed. With Ewald he conducted an experimental verification of the predictions of the latter's dynamical theory of X-ray diffraction. This and other X-ray experiments were carried out first in Berlin and later at the Technische Hochschule in Stuttgart, where he also made contributions on the diffraction of low-energy electrons and on the wave-mechanical theory of electrical contracts.

In 1933, with other refugees from the Nazis, he joined Professor Blackett's group at Birkbeck College, University of London, where he published papers on cosmic rays and neutron physics. Between 1936 and 1945 Ehrenberg worked at the EMI research laboratories, mainly on problems concerned with radar and the development of television tubes. Although possibly not so fruitful from a scientific point of view, this period of industrial research nevertheless gave him wide experience of electron beam devices and related techniques, which were to prove of considerable value in his later work. During this period he privately embarked on new studies; a paper on entropy and irreversible processes dates from this time, and thermodynamics became an abiding interest.

In 1945 Ehrenberg returned to Birkbeck as one of Professor Bernal's six Nuffield research fellows: his rôle was to apply electron optical and X-ray optical techniques to problems of crystallographic instrumentation, while remaining free to supervise research in other fields. During the next decade he built up a flourishing research group at Birkbeck College. For him and his students it was a period of intense and exciting activity. In 1949 he published, with R. E. Siday, a theoretical paper on electron optics in which they reached the surprising conclusion that electron interference can be affected by the presence of a magnetic flux even if the electron paths do not pass through a magnetic field This idea was independently put forward ten years later by Aharonov and Bohm and was then verified experimentally by R G. Chambers.

On the experimental side he contributed during these years to X-ray optics, particularly to the focussing of X-rays by total reflection at glancing angle from slightly curved surfaces, a development from his experiments in Berlin some 20 years before; but he found the usefulness of the technique to be limited by the excessively stringent requirements for smoothness, a difficulty that has recently been overcome by a group at the National Physical Laboratory, including his former student A. Franks. Another interesting development was the fine focus X-ray tube, devised by him in collaboration with W. E. Spear. The instrument was subsequently marketed by Hilger and Watts and found application in X-ray crystallography. It was characteristic of Ehrenberg that the royalties were assigned to a fund for the development of scientific instruments.

The immediate post-war years also marked the beginning of Ehrenberg's pioneering studies on the conductivity induced in insulating solids under electron bombardment, which eventually was to become his major concern. His 1954 paper with F. Ansbacher is still widely quoted and was one of the first detailed investigations in this field, which he and his collaborators continued to develop in subsequent years. His major work *Electronic Conduction in Semiconductors and Metals* was published in 1958 by Oxford University Press and contains the fruit of much original thought on the subject.

His appointment in 1951 to a Readership at Birkbeck College was followed in 1962 by a Chair of Experimental Physics; two years later, Ehrenberg became head of the Physics Department. Administrative responsibility thus came late in his career, but he applied to its problems the same penetrating and flexible mind that had enabled him to redirect his interests first from philosophy to physics and then successively between several fields of specialisation. After retirement in 1968 he continued to examine, to supervise research and to participate actively in colloquia. In his final years he brought to bear on the preparation of a book on *Cause, Necessity and Chanc* his combined knowledge of thr classical philosophy, science ar languages.

Werner Ehrenberg was a remarkable man, who showed great courage in facing and overcoming the many difficulties caused by his physical disability. His humanity, enthusiasm, experimental ingenuity and physical insight were an inspiration to all, and especially to his research students, who held him in deep affection for his total devotion to the search for truth and for the generosity and imagination with which he would enter, often at a moment's notice, into a discussion of almost any problem on the enquirer's terms. His friendship was as unforgettable as it will be irreplaceable.

Reports and publications

Philosophical Transactions of the Royal Society of London. A Mathematical and Physical Sciences. Vol. 280, No. 1298· A Discussion on Global Tectonics in Proterozoic Times. Organised by J Sutton, FRS, R. M. Shackleton, FRS, and J C. Briden Pp. 397–667 +plate 14 (London The Royal Society, 1976) UK £12, Overseas £12 50. [221

The Abortion Hurdle Race The Role of the Doctor as a Taker of Abortion Decisions By Francois Lafitte. Pp 10. (Solihull, West Midlands. British Pregnancy Advisory Service, Austy Manor, Wootton Wawen, 1975.) [261

Bulletin of the British Museum (Natural History) Zoology. Vol. 29, No 1 A Review of the Family Centropomidae (Pisces, Perciformes) By P. H. Greenwood. Pp. 1–81. (London. British Museum (Natural History, 1976) £5.85. [261

Physics and Ethics The Influence of Newton on Moral Philosophy By Professor D. D. Raphael. (Inaugural Lecture, 11 March, 1975) Pp. 17–23. 50p. Bonds and Bands By Professor N H March. Pp 25–35. (Inaugural Lecture, 29 April, 1975) Pp. 25–35 80p. (London· Imperial College of Science and Technology, 1975.) [261

University of London. University College Annual Report, 1974/1975 Pp. 156. (London. University College, 1976) [281

Philosophical Transactions of the Royal Society of London A· Mathematical and Physical Sciences. Vol 281, No 1299. Free Surface Oscillations and Tides of Lakes Michigan and Superior. By C H Mortimer and E. J Fee. Two Dimensional Normal Modes in Arbitrary Enclosed Basins on a Rotating Earth: Application to Lakes Ontario and Superior By D. B. Rao and D. J. Schwab A Theory of Short Period Tides in a Rotating Basin By P F. Hamblin. Pp. 1–111 (London The Royal Society, 1976) UK £6, Overseas £6 15. [291

Person to person

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